

The University of Chicago

THE EMBRYOLOGY OF THE GUINEA PIG

A DISSERTATION SUBMITTED TO THE FACULTY
OF THE DIVISION OF THE BIOLOGICAL SCIENCES
IN CANDIDACY FOR THE DEGREE OF DOCTOR
OF PHILOSOPHY

DEPARTMENT OF ZOÖLOGY

1935

By
JOHN PAUL SCOTT

Private Edition, Distributed by
THE UNIVERSITY OF CHICAGO LIBRARIES
CHICAGO, ILLINOIS

Reprinted from
AMERICAN JOURNAL OF ANATOMY, Vol. 60, No. 3, March 1937
JOURNAL OF MORPHOLOGY, Vol. 62, No. 2, March 1938
JOURNAL OF EXPERIMENTAL ZOÖLOGY, Vol. 77, No. 1, October 1937

The University of Chicago

THE EMBRYOLOGY OF THE GUINEA PIG

A DISSERTATION SUBMITTED TO THE FACULTY
OF THE DIVISION OF THE BIOLOGICAL SCIENCES
IN CANDIDACY FOR THE DEGREE OF DOCTOR
OF PHILOSOPHY

DEPARTMENT OF ZOÖLOGY
1935

By
JOHN PAUL SCOTT

Private Edition, Distributed by
THE UNIVERSITY OF CHICAGO LIBRARIES
CHICAGO, ILLINOIS

Reprinted from
AMERICAN JOURNAL OF ANATOMY, Vol. 60, No. 3, March 1937
JOURNAL OF MORPHOLOGY, Vol. 62, No. 2, March 1938
JOURNAL OF EXPERIMENTAL ZOÖLOGY, Vol. 77, No. 1, October 1937



THE EMBRYOLOGY OF THE GUINEA PIG

I. A TABLE OF NORMAL DEVELOPMENT¹

J. P. SCOTT

Whitman Laboratory, The University of Chicago

FIFTEEN FIGURES

CONTENTS

Introduction	397
Methods and materials	398
Effects of caesarian section	401
General features of development	403
Use and scope of the table	406
A table of normal development	408
Summary	431

INTRODUCTION

At the instance of Prof. Sewall Wright² the author began, in 1933, to investigate the embryological action of a guinea pig gene, *Px*, the most noticeable effect of which was the production of polydactyly. It was soon discovered that there was no account of normal guinea pig development which was accurately timed and extensive enough to permit comparison over the long period during which this gene acts. A new standard of comparison was developed, based on the most advanced animal in a litter. As a result, a body of material similar to that of Keibel's 'Normentafeln' was accumulated. These data, which should be of use to anyone attempting accurate embryological study of the guinea pig, are gathered together below.

¹ This paper includes the first part of a dissertation in zoölogy submitted to the faculty of the division of biological sciences of The University of Chicago in candidacy for the degree of doctor of philosophy.

² The author wishes to thank Professor Wright for invaluable aid and advice given during the progress of these researches.

METHODS AND MATERIALS

It is a common complaint of mammalian embryologists that the state of development of embryos within a single litter shows so much variation that it is impossible to describe them in terms of chronological age. There are two obvious causes for this: that a necessarily small sample must show a large standard deviation, and that the environments of two eggs developing in the same uterus are far more different than, for example, the environments of two sea urchin eggs developing in the same dish of sea water.

Mall ('18) has used size as a measure of development, but it is obvious (Draper, '20) that there is an enormous range of error. Somite number is also commonly employed, but has the disadvantages of covering only a relatively short period of development (14 to 20 days) and that the somites have only limited relationships with other organs and hence would not be expected to show a very high correlation with general state of development.

Inasmuch as time has been suggested as the unifying factor between various types and degrees of monstrosities in the guinea pig (Wright, '35), an attempt was made to obtain embryos with fairly accurate chronological ages. It was thought that the error inherent in this measure could partly be eliminated by using as a standard not the average state of development in a litter but that of the most advanced member, on the theory that there is a fairly sharp upper limit to the speed at which normal development can take place. Copulation was used as the then most accurate index of fertilization.

In agreement with Squier ('32) it was found that there is no reliable method of predicting the heat period in the female. The vaginal membrane may or may not be open before and during oestrus. The vaginal smear method gives a prediction accurate only within 2 or 3 days. Mated females almost invariably showed a vaginal smear of Stockard and Papanicolaou's stage I-II, but a smear of this type was not a reliable indication of mating behavior.

Copulations were therefore obtained by subjecting every female to the attentions of a vigorous male once per day, between the hours of 8.00 and 10.00 P.M. Matings were easiest to obtain at this time, when feeding had been completed and the building was quiet. Securing successful copulation was difficult unless the females were kept in their own pens and were used to being handled, although it is reported (Young et al., '35) that the copulatory reflex itself is not easily inhibited.

From 20 to 33% fewer matings were obtained than might have been expected on the basis of Young's tables of the frequency of the heat period. This is probably to be accounted for by certain difficulties in obtaining actual copulation, such as the lack of a sufficiently vigorous male (the sexual activity of the males is lessened for a day or so after copulation) or refusal of a female to submit to copulation even when in heat.

The mating behavior of guinea pigs has been sympathetically described by Draper ('20), Louttit ('27) and Young et al., ('35). Only minor variations from these accounts were observed. The moment of ejaculation is made unmistakable by the straining, bowed posture of the male, and in most cases by the resulting vaginal plug.

Because of a shortage of breeding females embryos were obtained by caesarian section, using ether anesthesia and nearly aseptic methods. From $9\frac{1}{2}$ days on the position of the embryo is marked by a swelling in the uterus. If the two-layered side wall of the uterus be cut at this point, gentle pressure will hull out the decidual mass, neatly divided on the anti-mesometric side by a ridge of decidual tissue at right angles to the long axis of the uterus. A small knot in the center of the ridge marks the center of the anti-mesometric side and the location of the embryo beneath (fig. 1). These landmarks disappear at later stages with the degeneration of the decidua capsularis. If the uterus is cut along the anti-mesometric side less bleeding results, but the embryo is almost certain to be injured. The chief post-operative difficulty was adhesion of uterus and intestines, probably caused

in part by clotting of blood which could not be entirely removed.

The decidual masses were immediately placed in cold Ringer's solution with a pin marking the anterior end. The mother was carefully sewed up and the embryos studied at leisure, sometimes being left as long as 1 hour in Ringer's solution before fixation. In most cases the outer extra-embryonic membrane (yolk sac) was removed and the living embryo studied under a dissecting microscope. The crown-rump length and placenta size were taken with a micrometer caliper at this time.

Most of the material was fixed in Bouin's fluid which, in spite of the slurs cast upon it by cytologists, is probably still the best micro-anatomical fixative. Others were fixed in 5% formol-Zenker; others in 10% formalin. A great part of the staining was done with Mallory's phospho-tungstic haematoxylin, in the hope that this highly differential stain would show progressive changes in histogenesis. A few embryos were stained in iron haematoxylin plus picro-indigo carmine, for mitosis counts. Up to $21\frac{1}{2}$ days the embryos were sectioned at 10μ , thereafter at 20μ , the sections being taken parallel to the roof of the myelencephalon or simply transverse in stages preceding flexure.

Before sectioning each embryo was drawn accurately to scale, each part being measured, and a complete written description made. The most advanced embryo of each litter was selected for study; in case of doubt the largest embryo was taken. After sectioning a written description was made from the slides with an idea of recording significant changes. Monstrous embryos from the same litters, to be described in a later paper, were studied at the same time.

The embryos were obtained from two different hereditary stocks in the Wright guinea pig colony: I, ID, and from random bred animals not related to the above. The origin of the semi-inbred I and ID stocks is described by Wright ('35). ID is the result of a cross between the I and D stocks, carrying, respectively, single factor and multiple factor polydactyly.

ID is therefore less inbred than I. Obvious differences between the two were the greater body size and greater development of atavistic digits in ID, the presence of brown coat and eye color in I, and the lack of black coat and eye color in the same. The white spotting and tortoise shell factors were present in both strains.

Embryos were taken at approximately 24-hour intervals from 10 to 30 days after copulation, with some extra litters at what appeared to be a critical period. A total of thirty-three litters was obtained from the inbred stocks and twenty-five from the random bred, including, respectively, 106 and seventy-five implanted embryos. It will be noted that the average is very close to three per litter.

Comparison showed that there was no obvious difference between development in the inbred and random bred stocks, and serious study was confined to the former. As matings were made always between animals heterozygous for the factor for polydactyly (Px), the animals described in the table are either $Pxpx$ or $pxpx$ except that below $18\frac{1}{2}$ days they may also be $PxPx$.

The age of the animals described is given in days, hours and minutes from the minute of ejaculation to the minute of incision into the female. It is estimated that the maximum range of error is 15 minutes. It is assumed that development practically ceased upon placing the embryos in Ringer's solution.

EFFECTS OF CAESARIAN SECTION

As many as three litters were taken from one female, depending on mating behavior. Some diminution of fertility resulted, as females bred within 2 months of an operation rarely became pregnant. However, one successful mating was obtained 21 days after an operation upon the uterus.

The resumption of the oestrous cycle after a terminated pregnancy showed marked regularity. Vaginal smears were taken daily from a number of convalescent animals. As shown by table 1, seventeen out of thirty-three cases showed a heat

smear at 4 or 5 days following the operation. The time of the second heat period, where recorded, was regular, occurring from 14 to 19 days after the first. In the eight cases where the first recorded heat period occurred at 19 days or over, it seems fair to assume that the real first period was overlooked. The figures in parentheses give the result of subtracting the time of the second observed period, or twice the second period, from the first.

TABLE 1

Time of first heat smear after successful caesarian. Pregnancy terminated at 10 to 30 days

<i>Days</i>	<i>Number of cases</i>
2	1
3	1
4	8
5	9
6	2
8	1
19 (4)	1
21 (6)	1
22 (6)	1
25 (6)	1
34 (4, 4, -)	3
43 (-)	1
Doubtful, 2 to 7	3
Total	33

It can be concluded that when pregnancy is terminated from 10 to 30 days following copulation the period of heat recurs at 4 to 5 days following the operation. No attempt was made to incite the animals to sexual behavior at this time, as the wounds were not healed. These observations are in harmony with those of Nelson ('33), who found that removal of fetal guinea pigs and their placentae brought on lactation in the mother. No observations were made upon lactation by the present author. There is no noticeable increase in the size of the nipples before 30 days. It is probable that the removal of the embryos produces an effect upon the pituitary, which in turn causes both lactation and oestrus, the action being humeral in each case.

The situation is not identical with the natural termination of pregnancy. It is commonly observed that female guinea pigs frequently come into heat within a few hours after parturition. Ovulation bears about the same relationship to heat period both in recently parous and virgin females (10 hours after parturition according to Lams, '13, or up to 1 hour after the end of oestrus according to Young et al., '35). It is possible that the delay of 4 to 5 days following caesarian section is caused by corpus luteum influence. Or post operative shock may be a factor.

GENERAL FEATURES OF DEVELOPMENT

Studies on the embryology of the guinea pig have been popular ever since Bischoff's general monograph (1852) raised the question of inversion of the germ layers. Later Lieberkühn (1882, 1884) discovered the notochordal canal, and most studies have been concentrated around these two problems. However, Lams ('13) and Squier ('32) have extensively studied the egg, while Harman and Prickett ('32, '33) have reported on the external form of the embryo. Numerous scattered reports upon the development of single organs also exist. Following is a short general account of development.

The cleaving egg of the guinea pig forms a typical mammalian hollow blastocyst which becomes implanted on the anti-mesometric side of the uterus. The blastocyst is oriented so that the inner cell mass is toward the mesometric side. As shown by Spee ('01), an implantation cavity is formed in the decidual tissue by erosion. Immediately upon implantation mitotic division ceases in the decidual tissue, though the latter subsequently shows great increase in size. The blastocyst gradually migrates away from the uterine lumen, leaving a trail of uterine epithelial cells behind it.

Since the publication of Sansom and Hill's latest paper ('31), all living authors except Maclaren (Maclaren and Bryce, '34) appear to agree that Selenka's (1884) interpretation of germ layer formation is the correct one. Bischoff's

(1852) original confusion of the trophoblast with entoderm was probably a result of the poor microscopical technique of the time, and Spee's ('01) and Maclaren's ('26) similar interpretations were probably caused by the use of fixatives such as Zenker's, which may cause violent shrinkage.

Entoderm is formed by delamination from the inner cell mass on the anti-mesometric side. The remaining mesectodermal portion of the inner cell mass (called ectoderm by most authors) becomes free from the overlying tr ager area of the trophoblast and migrates in the anti-mesometric direction, pushing the entoderm before it. The result is a two-walled vesicle, containing the germ ball (mesectoderm) at one end, and having both layers continuous with the tr ager at the other. The outer wall (trophoblast) degenerates, leaving the entoderm on the outside, and the tr ager hollows out to form the placental cavity. There are thus two elongated vesicles attached end to end, the entodermal vesicle partly enclosing that of the tr ager on its unattached side. This 'finger-like vesicle' stage is shown in figure 2.

Selenka's account of mesoderm formation was found to be incorrect by Huber ('18), whose account is confirmed by the present author. The germ ball hollows out to form the amniotic cavity, the amniotic membrane forming on the mesometric side, while the vesicle of the tr ager invaginates to form the placental cup. Notochord and mesoderm are formed from one side of the germ ball, thus establishing the long axis of the embryo. This has no constant relationship to the uterine axis. The mesoderm spreads out so as to line the entodermal vesicle, which then becomes homologous with the wall of the yolk sac of other forms. The amnion forms a pouch posterior to the notochord, and a thickening of mesoderm around this pouch forms the first indication of the allantois.

The yolk sac vesicle then expands, probably by imbibition of fluid, causing the germ ball to be flattened out into the embryonic shield (fig. 3). Invagination of the entoderm to form the gut takes place, together with the formation of a head fold. By torsion and flexion of the body, the embryo

comes to lie with its right side toward the mesometrium and placenta, and with its left side toward the yolk sac. The coelom is formed by splitting of the lateral plate mesoderm and opens out into the cavity of the yolk sac vesicle, which then becomes homologous with the extra-embryonic coelom of other forms, and within which the entire embryo in its amnion lies. The yolk sac mesoderm develops blood islands and later becomes highly vascularized. Especially prominent is the yolk sac artery, running straight to the edge of the yolk sac where its two branches form a circle above the placenta. The yolk sac in the guinea pig is thus open at the bottom, the edges being turned back over the embryo and attached to the *träger*. The allantois grows out from the pouch described above up through the extra-embryonic coelom and fuses with the placenta (*träger*), some time after the yolk sac circulation is established.

The germ layers in early development, except if approached through the *träger*, are certainly 'inverted' from the usual position, but the details of development make it certain that they are *not* transmuted and that the guinea pig, like other mammals, is descended from some form having a gastrula type of larva.

The allantoic diverticulum appears after the formation of the hind gut. Meckel's diverticulum is formed as an outgrowth of the gut some time after connection with the yolk sac has been severed. Subsequent to the establishment of the placenta (about 20 days) the pressure within the yolk sac vesicle is gradually relaxed, and after this the majority of embryos are found with heads directed toward the cervix. Villi are developed on the yolk sac adjacent to the placenta, but are free.

Unlike the pig the mesonephros remains in a primitive state, correlated with the type of placentation. Other general details of development are similar to those of other mammals, although the guinea pig embryo, as can be seen from the figures, has a distinctive appearance. Peculiarities of development of special organs do not lie within the scope of this paper.

Development of the guinea pig falls fairly naturally into five periods, divided by the following significant events (with approximate times): implantation (6 days), first somite (14 days), disappearance of anterior somites (21 days), and separate digits formed (28 days).

USE AND SCOPE OF THE TABLE

A new measure for development, namely copulation age in terms of the most advanced embryo reported for that age, has been used in the compilation of the table. Embryos of seventeen ages, at approximately 1-day intervals except one at $\frac{1}{2}$ -day intervals, were studied by the author. With the exception of two ages, 22-17-35 and 24-13-1 (omitted from the table), the result is a well-graded series. The most advanced embryo of 22-17-35 was found to be in almost exactly the same stage of development as 21-15-15. The single normal embryo of 24-13-1 was retarded nearly 2 days in development. The father of this litter produced other litters in which several embryos were similarly retarded.

The method thus gives fairly satisfactory results. The following types of errors are involved: 1) genetic, 2) inter-uterine, 3) descriptive.

The use of two related stocks has cut down some of the variation caused by genetic factors, and the description of accurately timed stages by other authors should further reduce it, provided the hypothesis of an upper limit to the speed of development is correct. However, genetic factors must be considered in making any comparisons with the table.

Intra-uterine errors are eliminated to some extent by the method of using only the most advanced embryo from a litter. Non-genetic inter-uterine differences may be caused by the following factors: 1) age of mother, 2) type of feed, 3) disease or accident to mother, 4) season of the year, 5) size of litter, 6) region of implantation, 7) proximity of embryos, and 8) ovulation time. Of these all but no. 6 are known to affect development to some extent. Of the first four, nos. 2 and 3 were fairly constant for the data described. Nos. 5, 6

and 7 are not controllable at the present time except by selection; this was not done on account of the difficulty of obtaining sufficient material.

According to Young et al. ('33) ovulation time for different female guinea pigs is practically constant within an hour with regard to the end of the oestrus period, but the period of oestrus may be as long as 18 hours ('35). Since under the breeding conditions copulations might occur with equal frequency at any time during oestrus, the average time between copulation and ovulation should be about half the average length of oestrus plus 1 hour, or approximately 5 hours. In individual cases the time is equally likely to be of any value from 1 to 9 hours (unless copulation should happen to be easier in one particular period of oestrus, which is possible), and this result is not seriously modified by allowance for the standard deviation of the oestrous period, 1.75 hours. Thus a range of error of about one-third of a day should rarely be exceeded. However, this is probably the most important single cause of error involved in the method. Now that methods for inducing oestrus and ovulation are known, future studies could be made on a basis of ovulation rather than copulation time.

An attempt was made to cut down errors of description by measurement, careful checking, and emphasis of stages where easily recognizable changes had occurred. However, it is impossible to determine the state of development by superficial observation. Comparison will be more exact if made under the same conditions as the present study: examination of whole embryos fixed but not cleared, and examination of sections made at a similar angle.

The changes undergone by the visceral arches and related parts of the face are the best external guides to state of development. The extra-embryonic membranes and placenta are better correlated with time, being unaffected by factors which slow up development of the embryo (these will go on developing normally for several days after the embryo has been experimentally destroyed), but there are few marked

morphological changes. As far as the data goes, size was found to be perfectly correlated with differentiation *within a litter*, the largest embryo being invariably the most advanced except in early stages of flexure. In later stages the relaxation of the body flexures increases the linear dimensions, adding apparent size to the most advanced embryos. Differentiation is also well correlated with somite number during the period when somites are being rapidly added.

Internally, the circulatory system was found to be the most useful, showing a long series of definite morphological changes. Histological changes in all organ systems were found to be of a gradual sort, difficult to mark off into stages. Data to be presented in part III of this paper indicate that the circulatory system is little affected by factors which produce marked changes in morphogenesis in other parts of the body.

Undated embryos compared with this table can be stated to have a minimum possible age of so much in terms of the table; and a maximum age of probably not over 2 days more, since no embryos were found to be retarded more than 2 days without showing obvious abnormalities. Draper's ('20) and Ibsen's ('28) tables may be consulted for size data on accurately-timed embryos.

A TABLE OF NORMAL DEVELOPMENT, BASED ON THE MOST
ADVANCED STAGE REPORTED FOR A GIVEN AGE

(Material included only where the time is accurate to the hour, except in early stages not studied by the author.)

I. Period of implantation

<i>Event</i>	<i>Time after copulation (days, hours, minutes) and parental stock</i>	<i>Author</i>
Sperm in first third of fallopian tube	0-1-	Hensen
Egg in second quarter of tube	0-3-	Squier
Two cells	0-23-	Lams
Four cells	1-6-	Squier
Egg enters uterus	3-8-	Squier
Eight cells	3-10½	Squier
Blastocyst	4-19-	Squier
Implantation	6-	Selenka, Maclaren, Sansom & Hill

II. Period of germ layer formation

<i>Event</i>	<i>Time after copulation (days, hours, minutes) and parental stock</i>	<i>Author</i>
Entoderm formed	6-	Selenka
Germ ball migrates; entodermal vesicle formed	6½-	Selenka
Träger cavity formed	6½-	Selenka
Maternal blood sinuses in placenta	8-	Selenka
Träger cavity fully expanded	8-	Selenka
Decidual tissue visibly swollen	9-	Hensen
Träger invaginated to form placental cup	9-	Selenka
Amniotic cavity formed	9-	Hensen, Selenka
Amniotic ectoderm differentiated	10-16-30 (C)	Scott
Projections of syncytial layer of placenta (villi) formed; uterus slightly eroded	10-16-30 (C)	Scott
Placental cavity obliterated except at rim of cup	11-12-	Hensen
Connection with uterine lumen lost	11-16-55 (I)	Scott
Germ ball split into mesoderm and ectoderm at one side	11-16-55 (I)	Scott
Mesoderm spreading from this point to cover amnion and part of entoderm	11-16-55 (I)	Scott
Allantoic pouch present	11-18-	Huber
Embryonic entoderm differentiated	11-18-	Huber
Notochord or head process formed	11-18-	Huber
Mesoderm lining more than half of entoderm	12-12-0 (I)	Scott
Entodermal vesicle begins to expand	12-12-0 (I)	Scott
Allantoic bud formed	12-17-13 (C)	Scott
Cone-shaped embryonic shield	12-17-13 (C)	Scott
Vesicle one-half expanded	12-17-13 (C)	Scott
Epithelial layer of placenta, with mesoderm, folded outward into sinuses (true villi)	13-11-12 (I)	Scott
Yolk-sac vesicle fully expanded to spherical shape, entirely lined by mesoderm	13-11-12 (I)	Scott
Mesoderm of yolk sac with blood islands	13-11-12 (I)	Scott
Peripheral entoderm forms epithelial layer around edge of placenta	13-11-12 (I)	Scott
Embryonic shield flat	13-11-12 (I)	Scott
Ectoderm and entoderm completely separated by mesoderm	13-11-12 (I)	Scott
Primitive groove present	13-11-12 (I)	Scott
Notochordal canal open	13-11-12 (I)	Scott
Notochord extends to anterior end of neural plate	13-11-12 (I)	Scott
Neural plate differentiated from rest of embryonic shield	13-11-12 (I)	Scott
Neural groove in cephalic portion of embryo	14-7-	Huber
Notochord a flattened plate fused with entoderm, no somites	14-7-	Huber

III. Period of formation of embryonic organs; embryos described by organ systems

Age 14-11-44, lower left (I), Scott (fig. 5)

- a. External structure.
 - 2. Body form. Elongate; head fold present.
 - 3. Somites. Two, with somite block.
 - 4. Nervous. Elevated brain plate, neural folds.
- b. Internal structure.
 - 1. Extra-embryonic membranes. Extensive projections of syncytial tissue; large eroded space in decidua.
 - 2. Somites, coelom. Myocoeles present; coelom present, divided behind, joined beneath head fold. Mesenchyme in head.
 - 4. Digestive. Fore-gut and oral plate formed.
 - 5. Circulatory. Separate heart primordia in ventral walls of coelom; two dorsal aortae present.

Age 14-11-, Huber

- a. External.
 - 3. Somites. Five.
 - 4. Nervous. Closed neural tube in one place.
- b. Internal.
 - 2. Somites. Notochord cut off entoderm anteriorly.

Age 15-12-45, lower right (I), Scott (fig. 6)

- a. External.
 - 1. Extra-embryonic. Allantois a pear-shaped sac hanging down into extra-embryonic coelom, as large as embryo proper.
 - 2. Form. Lying with left side on yolk sac; cephalic flexure about 90°; rest of body uniformly curved; allantois projects from posterior end of body.
 - 3. Somites. Thirteen.
 - 5. Sense organs. Small optic elevation. Wide open otic vesicle.
 - 6. Visceral arches. Small mandibular arch without knob; open mouth; large square hyoid arch; wide open cervical sinus.
 - 8. Circulatory. Large heart primordium visible.
- b. Internal.
 - 1. Extra-embryonic. Maternal sinuses divided, small; strands of syncytial tissue extend almost to edge of compact decidua; epithelial layer of placenta breaking down; mesodermal layer has a reticular portion filling a central pit. Yolk sac highly vascularized but circular artery incomplete; marginal cells contain very large vacuoles; sub-placental yolk sac loose and irregular. Allantois contains scattered mesenchyme, fluid, and numerous blood vessels.
 - 2. Somites. All but last three with medial wall broken down. No notochordal canal; notochord separate from gut except posteriorly and anteriorly, at latter point continuous with roof of pharynx. Coelom open into extra-embryonic coelom for first time. No ventral mesentery to hind gut. Transverse septum established; pericardial and peritoneal coeloms join dorsally.

3. Nervous. Telencephalon short, unpaired. Diencephalon strongly compressed, with large lateral optic vesicles, touches oral portion of hypophysis. Mesencephalon short, not prominent. Myelencephalon with thick roof, four neuromeres behind nerve V, fourth being large. Otic vesicle opposite second and third. Nerve cord open at posterior end. Anlagen of ganglia V, VII-VIII present.
4. Digestive. Hind gut present, two pharyngeal pouches.
5. Circulatory. Two-chambered heart with dorsal atrium, ventral ventricle; looped to left side; with muscle fibers. Two aortic arches, hyoid the largest, mandibular with an anterior carotid loop passing near the hypophyseal region of the diencephalon. Large left and very small right yolk-sac arteries behind posterior intestinal portal; right dorsal aorta ends in two branches to the allantois. Large right and small left allantoic veins running along fold in lateral body wall which gives rise to amnion; joined in front of anterior intestinal portal by large paired yolk-sac veins; small and irregular anterior cardinals run from transverse septum as far as otic vesicle. Nucleated blood cells in vessels; both embryonic and extra-embryonic circulation established.
6. Excretory. Intermediate cell mass swollen on somites 10 to 13.
7. Endocrine. Wide open pit touches diencephalon in front of notochord (Rathke's pouch).

Age 16-12-6, upper left (ID), Scott (fig. 7)

a. External.

1. Extra-embryonic. Yolk sac with well-developed artery and veins. Allantois attached to placenta.
2. Form. Body longer; tail bud behind allantois. Three divisions of brain slightly marked.
3. Somites. Twenty-three, long somite block.
4. Nervous. Roof of fore- and mid-brains thin in center. Thin swollen roof of myelencephalon visible. Spade-shaped forebrain; head with two anterior lobes.
5. Sense organs. Optic elevation transparent, with indication of chorioid fissure. Transparent otic vesicle above the posterior part of the hyoid; small exterior opening. Indication of ganglion V above mandibular arch.
6. Visceral arches. Mouth wide open, with knobbed mandibular processes hanging down on either side, these not touching head. Cervical sinus slightly sunken, contains third and rudiment of fourth arches.
7. Limb buds. Forelimb present as narrow thickening at base of somites 5 to 10.
8. Circulatory. Heart seen beating externally; on left a large ventricle continuous with atrium, on right coiled ventral aorta from ventricle. Anterior cardinal vein visible.

b. Internal.

1. Extra-embryonic. Placenta with epithelial layer of central pit folded out into the extra-embryonic coelom, carrying maternal sinuses; placenta separable from decidua on edges along line of sub-placental yolk sac, which is now broken down into separate cells. Yolk sac highly vascular, thrown into folds over blood vessels near placenta. Attachment of allantois superficial only.

2. Somites. Anterior show convex dermatome lined with core cells. Dorsal mesentery of the heart persists only for short distance in front of transverse septum.
3. Nervous. Telencephalon oval in section, without lobes. Diencephalon with rounded roof; thick throughout; posterior pouch meeting Rathke's pouch. Mesencephalon nearly round, with two ventro-lateral thickenings (nuclei N. III). Metencephalon with thick wall, one neuromere in front of ganglion V. Myelencephalon with thick walls, five prominent neuromeres behind ganglion V, fifth the largest; thin roof extends back of fifth neuromere.

Ganglion V oval, touches metencephalon and outer ectoderm. Ganglion VII-VIII a smaller, rounded mass ventral to the otic vesicle. Very small ganglion IX behind the otic vesicle.

Nerve cord entirely closed, a narrow tube with side walls thickened, except posteriorly.

Otic vesicle round and thick walled. Optic vesicle also thick walled, extends dorso-laterally from the base of the diencephalon, flattened where it touches the outer ectoderm, thinnest at dorsal tip. Nasal plate of thickened ectoderm on the anterior ventral corner of the head.

4. Digestive. Maxillary process represented by a ridge ventral and partly lateral to eye, covering about half of it, with an ectodermal thickening on the corner of the head. Knobbed mandibular processes joined ventrally and posteriorly.

Hyomandibular pouch with long dorsal pocket. Pouches 1 to 3 touch outer ectoderm; no perforation. Fourth pouch shallow.

Posterior pharynx with four corners. Esophagus-trachea compressed, slightly widened at anterior end of transverse septum. Omental bursa in the transverse septum, parallel to stomach enlargement. Solid mass of liver tissue ventral to gut at posterior end of transverse septum. Long narrow diverticulum in yolk stalk. Gut ends blindly in the tail bud, touching the ventral ectoderm and continuous with undifferentiated tissue.

5. Circulatory. Heart with posterior loop, large atrium on right, smaller ventricle on left, ventral aorta ventral to atrium.

Three aortic arches, hyoid the largest, mandibular runs to carotid only. Dorsal aortae joined at somite 8 and behind. Large yolk sac artery on left of diverticulum, dividing into two in the yolk stalk, right branch joined by small right yolk sac artery. A small artery to left side of gut anterior to this. Short paired portions of aorta in tail bud behind allantoic arteries.

Walls of pericardium proliferating medial to anterior cardinals, which reach fore- and mid-brains. Right duct of Cuvier larger than the left, which empties into it. Yolk sac veins enter from in front of diverticulum, run lateral to gut and dorsal to liver, entering ducts of Cuvier. Post cardinals present, very small.

6. Excretory. Mesonephric duct in lateral part of urinary ridge, fused with ectoderm in tail bud and opening into coelom at somite 10. Open pronephridial tubules on somites 10 to 16, 12 and 13 with two.

7. Endocrine. Thyroid anlage present as thickening of basal-posterior wall of hyomandibular pouch. Rathke's pouch now within mouth.
8. Limb buds. Fore limb bud a fold and slight thickening of mesenchyme at dorsal corner of coelom, with slight thickening of ectoderm ventral to it.

Age 17-12-40, lower right (ID), Scott (fig. 8)

a. External.

1. Extra-embryonic. Allantois fused to placenta.
2. Form. Slight cervical flexure, mandibular processes touching head and body. Tail thick, knobbed.
3. Somites. Twenty-nine, short somite block.
4. Nervous. Five divisions of brain visible, telencephalon rounded.
5. Sense organs. Eye touched by maxillary process, horseshoe shaped.
6. Visceral arches. Mandibular process large, rounded, maxillary process about half the length. Hyoid two lobed. Fourth visceral arch in cervical sinus.
7. Limb buds. Tip of fore limb bud slightly curved ventrally; hind limb bud indicated but not discrete.
8. Circulatory. Post cardinal visible; branches of anterior cardinal present on mesencephalon.

b. Internal.

1. Extra-embryonic. Allantois covers placenta with thin membrane, fused only at edges, no interpenetration with placenta, ventral membrane of allantois gone. Placenta a spongy network of syncytial tissue containing small maternal sinuses. Open space beneath containing leucocytes and cell debris.
2. Somites. None entire, first almost disappeared. Notochord arises from undifferentiated area in tail bud, free for short distance but surrounded by mesenchyme from somite 20, very small over pharynx, ends behind hypophysis, nowhere connected with gut. Convex dermatomes begin at somite 11.
3. Nervous. Telencephalon somewhat heart-shaped, with expanded dorso-lateral portion, thickened walls. Diencephalon with rounded roof continued a short distance dorsal to telencephalon; posteriorly with thin floor and three angles to roof; posterior pocket present.
Otic vesicle thin walled, opposite neuromere 3. Optic vesicle with thickened retinal layer, lens placode. Nasal plate thick, extensive, shows very slight invagination at corner of head.
Ganglia VII and VIII distinguishable, former sends ventral round branch to hyomandibular cleft. Petrosal ganglion present, nerve to second visceral pouch. Jugular ganglion X connected with neuromere 5, small branch to 4. Also branch to ganglion XI along the side of the myelencephalon. Ten dorsal root ganglia present, first opposite somite 4.
4. Digestive. Ectodermal thickening of maxillary process is ventral, opposite similar mandibular thickening; mesenchymal thickening lies between ectodermal thickening, lens plate and ventral side of eye.

Tracheal portion of the esophagus enlarged and rounded at level of somite 11, forms short diverticulum below atrial mesentery. Stomach area defined. Liver with right and left dorsal lobes projecting into coelom. Dorsal pancreatic diverticulum at posterior end of liver. Thin-walled, blind, ventral allantoic diverticulum, about twice as long as depth of gut at this point. Behind this the anal plate.

5. Circulatory. Atrium with right and left anterior blind pockets in front of ducts of Cuvier. Large sinus venosus formed below and in front of liver, all vessels entering it. Four aortic arches, third largest, only three and four connect with dorsal aortae. Right yolk sac artery anterior to left, runs to mesentery only.

Largest branch of anterior cardinal runs superficially above the eye. Large post cardinal in nephric ridge, smaller behind fore limb bud. Yolk sac veins break up in liver, as do allantoics, which run to the posterior edge of the allantoic stalk, where body wall and allantois are fused.

6. Excretory. Pronephric tubules on somites 10 to 12, 11 and 12 with two. Otherwise two coiled mesonephric tubules per segment except last two somites, where intermediate cell mass present. Tubules connect to mesonephric duct, which ends close to hind gut and ectoderm in anal plate region.
7. Endocrine. Thyroid shows open diverticula from floor of mouth. Rathke's pouch closed posteriorly.
8. Limb buds. Fore limb bud rounded in section, of considerable thickness and undercut ventrally, where there is a thickening of ectoderm. Somites touch base of bud. Posterior bud only an indefinite thickening of posterior coelomic walls.

Age 18-12-15, lower right (ID), Scott (fig. 9)

a. External.

2. Form. Cervical flexure present, head touching heart. Sacral flexure also present.
3. Somites. Thirty-one visible.
5. Sense organs. Nasal pit present. Eye pigmented in black-eyed animals. Lens cup formed.
6. Visceral arches. Maxillary process continuous with lateral wall of nasal pit.
7. Limb buds. Hind limb bud defined.
8. Circulatory. Allantoic vein visible.

b. Internal.

1. Extra-embryonic. Placenta not studied.
2. Somites. Thirty-three, the first two largely broken down, the last three entire. Vesicular nuclei in myotome to somite 11. Dermatome convex from somite 18. Pericardial and pleural coeloms partly separated along line of anterior cardinal veins; vacuolated cells in this region.

3. Nervous. Marginal layer developed in myelencephalon; intermediate layer near cranial nerve roots and in ventral part of cord anteriorly. Telencephalon bilobed anteriorly, heart-shaped behind, the ventral portion being narrow. Diencephalon with large ventral and small dorsal posterior pockets. Cavity of mesencephalon with ventral groove; thin point in roof. Metencephalon with two neuromeres in front of nerve V.

Nasal plate with shallow pocket, is continuous with ventral ectodermal thickening of maxillary process. Optic cup formed, with chorioid fissure, vascularized. Otic vesicle flattened adjacent ganglia VII-VIII.

Ganglion V does not touch ectoderm; fibrous mandibular branch; nucleated maxillary and ophthalmic branches; latter ends above eye. Ganglion VII distinct, with hyoid branch. Ganglion X extends to third and fourth visceral arches. Nerve XI extends to fourth spinal root. Traces of XII roots on ventral side of myelencephalon. Twenty-one dorsal root ganglia; first small; twelve well formed and globular.

4. Digestive. Hyoid arches produced in V-shaped ridge in floor of mouth; roof of mouth with lateral grooves. Second and third arches also show ridges in floor of mouth. Ectodermal pouch above corner of mouth.

Posterior pharynx and esophagus with ventral tracheal expansion, cut off at somite 7; right and left bronchi present. Stomach large, compressed, inclined to left with omental bursa on right. Mesentery elongate and folded to the left behind bursa; expanded in region of yolk sac veins. No yolk sac diverticulum; gut expanded posterior to yolk stalk.

5. Circulatory. Small septum to right of opening of sinus venosus; small anterior inter-atrial septum.

Fourth aortic arch the largest; first and second represented by ventral branch off the third. Allantoic arteries given off at somite 30 medial to wolffian duct. Paired caudal aortae.

Vein from yolk sac single but divides over top of gut into large right and small left branches which run forward. Common cardinals large. Anterior cardinals receive branches from base of visceral arches, spread out superficially in region of diencephalon. Post cardinals extend to somite 29, with small branches to tail.

6. Excretory. Open pronephric tubules scattered from somites 10 to 19, total ten. All tubules stop about somite 27, posteriorly a solid mass. Mesonephric duct touches gut back of hind limb bud.
7. Endocrine. Thyroid closed off gut, closely applied to root of first two aortic arches. Small aortic bodies, extending to about somite 12. Slight folded area (infundibulum) on bottom of diencephalon.
8. Limb buds. Fore bud with plane lateral and medial surfaces; considerably undercut; ectodermal thickening at tip. Somites 11 and 12 still extend to bud; base touched by spinal nerves. Posterior bud rounded, with ectodermal thickening at top; nerves not developed.
9. Skeletal. Anlagen of centra and neural arches present; centra distinct to about somite 10.

Age 19-2-45, second left (I), Scott (fig. 10)

a. External.

2. Form. Maximum cervical and sacral flexures, respectively about 135 and 180°.
3. Somites. Thirty-five visible.
4. Nervous. Roof of myelencephalon extends nearly halfway to tip of visceral arches.
5. Sense organs. Elongate nasal pit.
6. Visceral arches. Maxillary process nearly as long as mandibular. Hyoid not bilobed. Deep cervical sinus.
7. Limb buds. Fore bud elongate in ventral direction; hind with distinct ventral edge.

b. Internal.

1. Epithelial layer of placenta largely disintegrated around the edges; the syncytial layer has penetrated the embryonic mesenchyme and produced a spongy tissue, alternate holes being filled with maternal blood and embryonic (non-maternal) mesenchyme containing embryonic blood vessels. Two systems are then separated by syncytial cells plus walls of blood vessels.
2. Somites. Thirty-five, with vesicular nuclei in myotomes to about somite 14. Notochordal sheath vacuolated in anterior portion.
3. Nervous. From diencephalon back a distinct and in some places thick marginal layer. Walls of telencephalon thickened ventrally; bilobed posteriorly. Diencephalon in section tongue-shaped with expanded tip; thin floor.

Long, deep nasal pit, closed at posterior end; maxillary process reaches posterior end. Otic vesicle with flat pocket (utriculus) lateral to endolymphatic duct.

Nerve III passes as far as maxillary process. Ganglion nodosum of X present. Nerve XII passes as far as swelling behind cervical sinus. Trace of nerve I. Fifth and sixth spinal nerves anastomose. Twenty-four dorsal root ganglia visible.

4. Digestive. Trachea bifurcates at somite 6; bronchi about one somite long. Laryngeal groove in third visceral arch, with lateral thickening. Thickening in mesentery to left of enlarged portion of gut behind yolk stalk.
5. Circulatory. Anterior atrial septum large; left ventricle larger than right. Fifth aortic arch present. Segmental arteries given off dorsally by aorta. Allantoic arteries with two roots, one lateral to wolffian duct. Arteries to fore limb bud at somites 9, 10. Carotids pass ventral to otic vesicle.

Sinus venosus anterior to liver, reduced, partly replaced by post cava, which lies on right. Left allantoic vein enters liver. Single yolk sac vein throughout, passing dorsal to gut and forward parallel to it on the right.

6. Excretory. Only two open tubules, at somites 11 and 12. Mesonephric duct enters gut above allantois, is expanded near thickening of mesenchyme anterior to this (anlage of metanephros).
7. Endocrine. Aortic bodies larger. Large, pear-shaped infundibulum.
8. Limb buds. Hind limb bud has ectodermal thickening on tip.

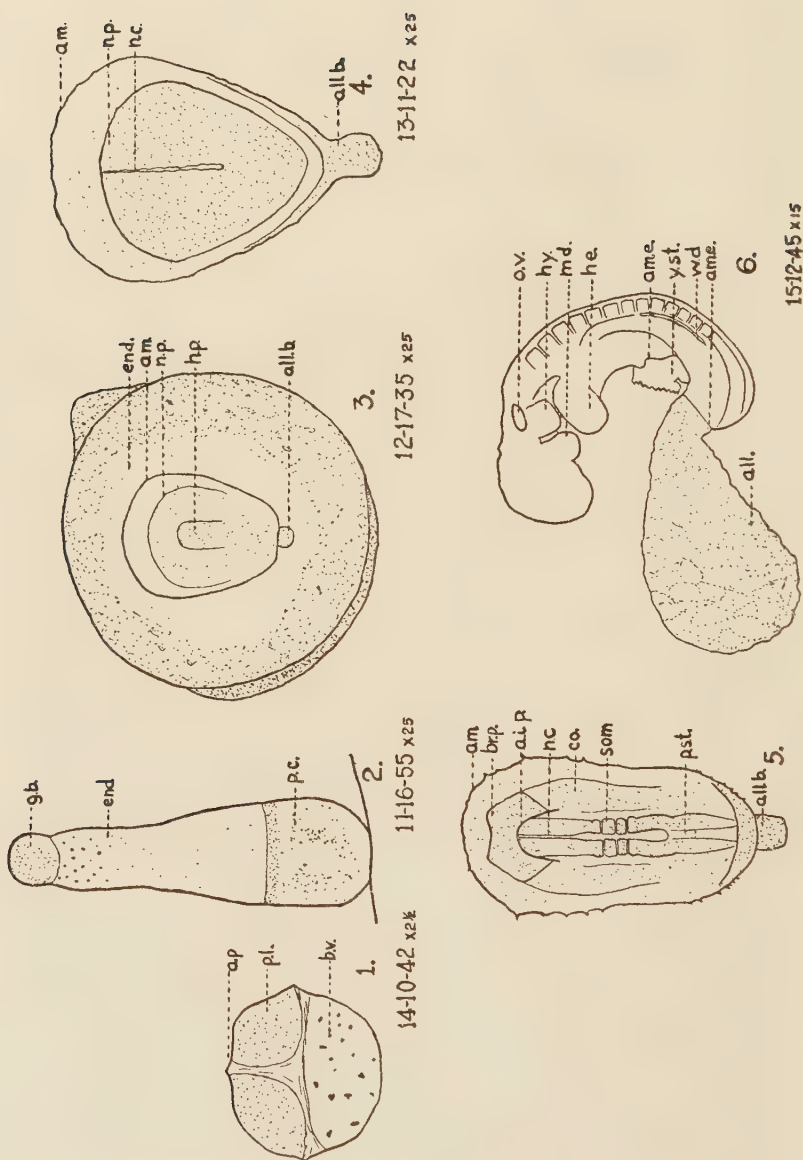
Age 19-16-55, middle left (ID), Scott (fig. 11)

a. External.

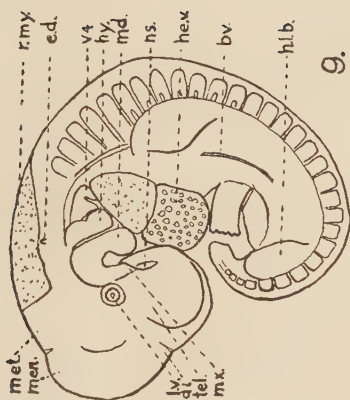
1. Extra-embryonic. Yolk sac villi developed around edge of placenta.
2. Form. Cervical flexure about 120°. Tail pointed.
3. Somites. Thirty-eight visible, extending to near tip of tail.
5. Sense organs. Lens cup closed.
6. Visceral arches. Lower end of hyoid pointed; cervical sinus half closed; third the last visible visceral arch.
7. Limb buds. Hind limb bud elongate in ventral direction.

b. Internal.

1. Extra-embryonic. Not studied.
2. Somites. Thirty-eight, vesicular nuclei scattered generally throughout myotomes. Convex dermatome from somite 20. Anterior notochordal sheath fibrous. Allantois now continuous with left body wall, not with right, constricting opening into extra-embryonic coelom. No ventral mesentery behind the pancreas. Pericardial and pleural cavities continuous only at extreme anterior end. Umbilical pouch present, without posterior wall.
3. Nervous. Telencephalon with marginal layer; intermediate layer developed in corpora striata, walls of diencephalon, metencephalon, myelencephalon and dorsal part of cord; dorsal sensory (oval) tract in cord present. Telencephalon with ridge in ventral floor leading to corpus striatum; lobes enlarged. Diencephalon with thick lateral walls; basal portion of tongue-shaped lobe thin walled. Metencephalon with deep ventral pocket. Nasal pit narrow; maxillary process lateral to posterior portion. Optic cup well formed; lens vesicle free from ectoderm. Utriculus with shallow posterior ventral pit (indication of cochlea).
Nerve III passes to above eye. Ophthalmic branch of V with lobe at base, passes slightly beyond eye; maxillary and mandibular branches pass to ends of respective processes; lower portion of maxillary fibrous. Ganglion nodosum (distal ganglion) of X in fourth visceral arch. XII with five or six large roots. Twenty-five well-formed dorsal root ganglia.
4. Digestive. Mandibular processes broadly joined, with anterior groove. Mouth cavity shallow and wide. Fourth visceral pouch touches ectoderm, sends long posterior pocket back below last aortic arch.
Trachea and bronchi about $1\frac{1}{2}$ somites in length. Esophagus where separated very small. Stomach expanded in center. Gut bends sharply into bottom of umbilicus, where it runs straight back, slightly on the left side; greatly enlarged on bottom of umbilicus. Dorsal and ventral lobes of liver present. Bile duct and pear-shaped gall bladder in right ventral body wall behind stomach. Allantoic diverticulum extends almost to umbilicus.
5. Circulatory. Interventricular septum surrounds foramen on all sides. Interatrial septum perforate anteriorly and incomplete posteriorly. Atria with posterior lobes (auricles). Common opening of post cava and right common cardinal has sleeve valve.

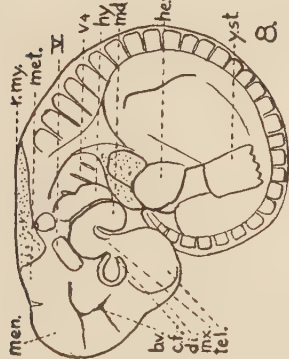


Figures 1 to 6



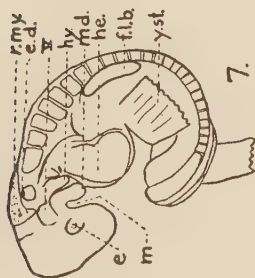
9.

18-12-15 x10



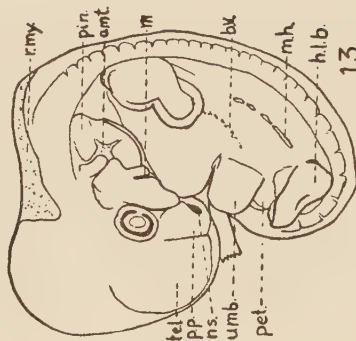
8.

17-12-40 x10



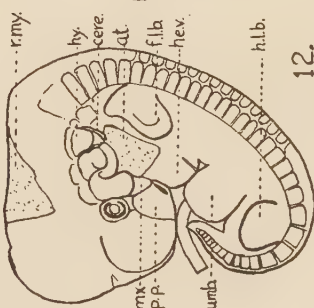
7.

16-12-6 x10



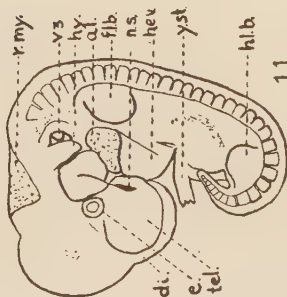
13.

21-15-15 x5



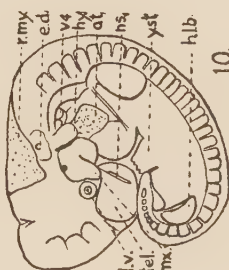
12.

20-17-10 x5



11.

19-16-55 x5



10.

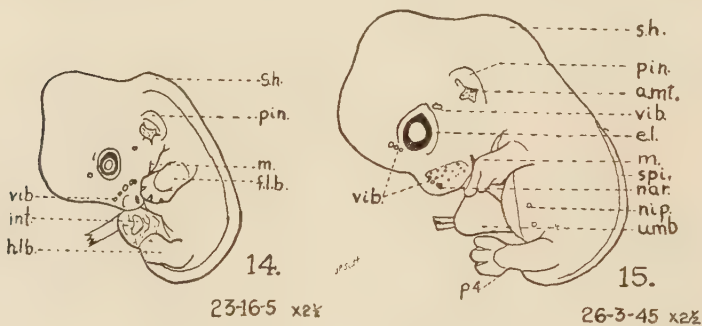
19-2-45 x5

Figures 7 to 13

Lumen of the aorta constricted. Allantoic arteries with external branches only; irregular branches to hind limb bud at same point.

Small right allantoic vein joins the large left in a considerable sinus behind liver; liver sinuses large, allowing free passage of blood; yolk sac vein smaller than left allantoic; left common cardinal runs a long distance posteriorly and loops back to join the post cava. Anterior cardinal with large sinus on head behind diencephalon. Paired caudal veins with irregular connections to post cardinals.

6. Excretory. Two open tubules on somites 10 and 11. Primitive Bowman's capsules present, with thick wall and no capillaries. Urinary ridge a rounded projection at the base of the mesentery, extending laterally from the aorta.



Figures 14 and 15

7. Endocrine. Cavity of Rathke's pouch entirely closed off; cellular connection with oral ectoderm remains.
8. Limb buds. Fore bud flattened laterally near tip; ectodermal ridge large; marginal vein present; all adjacent somites broken down. Hind bud undercut, rounded at tip.

Age 20-17-10, lower right (ID), Scott (fig. 12)

a. External.

1. Extra-embryonic. Amnion includes allantoic stalk; edge of yolk sac constricted. Coelom closed.
2. Form. Angle at end of myelencephalon; tip of tail turned up; prominent umbilical pouch.
3. Somites. Thirty-nine visible.
5. Sense organs. Nasal pit small, wide premaxillary process. Eye prominently pigmented; haemal ring.
6. Visceral arches. Maxillary process longer than mandibular; mandibular and hyoid arches subdivided; cervical sinus a slit; hyoid the last visible arch.
7. Limb buds. Slight indication of hand.

EXPLANATION OF FIGURES

- 1 Lateral view of freshly removed decidual capsule aged 14-10-42.
- 2 Germ ball stage, lateral view. I stock.
- 3 Partly expanded embryonic vesicle showing ventral (external) side of embryo. Cross bred stock.
- 4 Embryonic shield, in same position. I stock.
- 5 Two-somite embryo, in some position. I stock.
- 6 to 15 Series of embryos described in text, from left side. Figures 6, 10 from I stock; 7, 8, 9 to 15 from ID stock.

ABBREVIATIONS FOR ALL FIGURES

a.i.p., anterior intestinal portal	mes., mesencephalon
all.b., allantoic bud	met., metencephalon
am., amnion	m.h., milk hill
a.m.e., edge of amnion	mx., maxillary process
a.mt., external auditory meatus	nar., nostril
a.p., antimesometric pole	n.e., notochordal canal
at., atrium	nip., nipple
br.p., brain plate	n.p., neural plate
b.v., blood vessel	ns., nasal pit
cere., cervical sinus	o.v., otic vesicle
c.f., choroid fissure	p ₄ , fourth point on limb bud
co., coelom	p.e., placental cup
di., diencephalon	pe.t., perineal tubercle
e., eye	pin., pinna
e.d., endolymphatic duct	p.l., pink lobe
e.l., eyelid	p.p., premaxillary process
end., entoderm	p.st., primitive streak
f.l.b., fore limb bud	r.my., thin roof of myelencephalon
g.b., germ ball	s.h., shoulder hump
he., heart	som., somite
he.v., ventricle of heart	spi., spinal ridge
h.l.b., hind limb bud,	tel., telencephalon
h.p., head process	umb., umbilicus
hy., hyoid arch	v ₃ , v ₄ , third and fourth visceral arches
int., intestine	vib., vibrissa
l.v., lens vesicle	w.d., wolffian duct
m., mouth	y.st., yolk stalk
md., mandibular process	V, trigeminal ganglion

b. Internal.

1. Extra-embryonic. Not studied.

2. Somites. Forty (maximum number found at one time), the last three very small; convex dermatomes from twenty-five; dorsal edge of dermatome not broken down from eighteen. Notochord shows fibrous sheath to about somite 30. Umbilical opening slightly constricted; very small opening on right into extra-embryonic coelom of stalk. Pleural folds developing above and below, but not joined; pleural cavities joined ventrally anterior to veins into heart; here continuous with pericardial cavity; smaller opening on right on account of right common cardinal.

3. Nervous. Mesencephalon with intermediate layer. Telencephalon not heart shaped; in front of center of foramen of Monro the roof of the diencephalon partly invaginated, the corners produced (future chorioid plexi) extending into the lateral ventricles; round peak between them. Mesencephalon shows fold at entrance to metencephalon, whose walls are straight above, curved in middle, and nearly transverse below. Heavy walls of myelencephalon approximated ventrally.

Shallow groove between nasal pits, which contain a secondary lateral pit, are closed from tip of maxillary process, ending blindly. Eye with thick retinal and thin chorioid layer; lens cells indicated in inner layer; lens cavity half obliterated; eye projects out of head about one-third. Endolymphatic duct separated from ectoderm; utriculus elongate, grooved above level of nerve (anlage of canals), cochlear pocket descends to level of notochord.

Nerve III passes to anlagen of eye muscles. Ganglion VII-VIII a large oval mass chiefly adjacent to cochlear pouch. Separate root for VII, which has short nerve. Nerve IX to hyoid arch. Nerve X passes back parallel to trachea and half its length. Nerve XII, accompanied by branches from anterior spinal nerves, reaches lower ganglion of X. Thirty-one distinct dorsal root ganglia.

4. Digestive. Maxillary process undercut dorsally and rotated about 30° medially, producing a broad, shallow groove in center. Small medial ridge near tip of lower jaw (incisor anlagen), and small tongue ridge posteriorly. Hyoid small below, large above. Posterior part of mouth with deep groove in roof. Cervical sinus with narrow entrance; contains two reduced visceral arches. Hyomandibular pouch (Eustachian tube) extends dorsally to level of ganglion VIII; fourth pouch does not connect with ectoderm.

Trachea cut off behind fourth pouch; mesenchyme concentrically arranged around esophagus. Stomach inclined about 60° to left; nearly half as wide as body cavity. Bile duct branched. Pancreas long, no lumen, in mesentery posterior to stomach. After entering umbilicus gut is coiled forward, then down to bottom and over to left before meeting enlarged portion; from latter short Meckel's diverticulum runs over to left. Large allantoic diverticulum extends into allantoic stalk. Anal plate in perineal tubercle in front of tail.

5. Circulatory. Pulmonary vein enters left atrium behind division of trachea.

Lumen of aorta constricted into right and left portions, not cut off; these rotated to right passing anteriorly, become irregular. Pulmonary aorta given off on left before end of pericardium; branches join dorsal aortae behind fourth pouch; left branch largest. Systemic branches given off in front of fourth pouch; ventral aorta splits below thyroid; branches to hyoid and third visceral arch; hyoid breaks up, other joins dorsal aorta near ganglion nodosum X. Dorsal aortae (carotids) join in front of diencephalon. Single arteries to fore limbs at somite 10.

Left common cardinal enters right atrium. Precardinals enlarged in front of heart, above ear, and medial to ganglion V; subclavians received at somite 10. Post cardinals inconsiderable in size after giving off irregular branches to hind limb buds.

Both embryonic and non-nucleated corpuscles in blood. Large arteries with definite connective tissue in walls.

6. Excretory. Posteriorly the two urinary ridges form a round mass enclosing the aorta. First tubules at somite 12; only open ones at 13. Metanephric duct from wolffian at somite 33; small metanephric vesicle at 32; opening of wolffian duct into allantoic region on a papilla.
7. Endocrine. Infundibulum larger than oral portion; walls wrinkled. Aortic bodies (anlagen of sympathetic ganglia, etc.) show branches to spinal nerves; reduced after somite 15.
8. Limb buds. Fore bud solid mesenchyme; entered by nerves separately from ganglia 5 to 10 (somites 8 to 13). Hind bud with marginal vein; nerves enter base of bud from ganglia 23 to 29.
9. Skeletal. Centra distinguishable to about somite 33.
10. Genital. Small genital ridge on medial side of urinary ridge from somites 13 to 16. Primordial germ cells in thickened epithelium at this point; thickening continues posterior to ridge.
11. Muscular. Eye muscle anlagen present as mesenchymal thickenings medial and posterior to eye.

IV. Period of formation of adult organs (ganglion 1 = somite 4)

(All stages described by the author. Extra-embryonic membranes and placenta not studied in this group.)

Age 21-15-15, lower right (ID) (fig. 13)

a. External.

2. Form. Cervical flexure slightly less than 90°, head roughly square, hind brain sunken. Milk hills present.
3. Somites. Anterior somites indistinct.
4. Nervous. Divisions of brain not externally distinct.
5. Sense organs. External auditory meatus indicated.
6. Visceral arches. Maxillary process broad; mandibular into anterior narrow jaw and two posterior eminences in front of ear.
7. Limb buds. Hand flattened; proximal portion of fore limb round; foot formed on hind limb bud.

b. Internal.

2. Somites. Twenty-seven to forty-two distinguishable, last occupying the extreme tip of the tail bud; dorsal edge of the dermatome entire from somite 30 posteriorly. Fibrous sheath of the notochord to about somite 32. Umbilical pouch with narrow neck. Pleural folds complete anteriorly for short distance.

3. Nervous. Intermediate and fibrous layers on roof of diencephalon. Lobes of telencephalon hemispherical; foramen of Monro defined; chorioid plexi of lateral ventricles thin walled; posterior lobes with thin medial wall. Anterior lobe of diencephalon an inverted V between the hemispheres; walls thick; narrow third ventricle. Cavity of mesencephalon reduced to an oval. Myelencephalon shortened by reason of alteration of flexure, expanded at anterior end; neuromeres reduced. Cord wider ventrally than dorsally.

Nasal pits with median diverticulae (Jacobsen's organs); separated by anlage of septum. Chorioid and retinal layers of eye fused; optic canal nearly closed; fibers in optic stalk; lens cavity obliterated by lens cells running medio-laterally. Endolymphatic duct long; end expanded.

Nerve I shows large masses of nucleated tissue between hemispheres and nasal pits. Ganglion V vascularized, subdivided into bundles; ophthalmic and maxillary branches pass to nasal region. Ganglion VII contains definite fiber paths. Nerve IX passes to ventral side of pharynx. Vagus nerves unite beneath the esophagus at the bifurcation of the trachea and break up into branches at the anterior end of stomach. Nerve XII separates from vagus to go to base of tongue. Thirty-four dorsal root ganglia, last very small.

4. Digestive. Maxillary processes rotated about 45°. Roof of mouth arched; at level of hypophysis folds hang down. Mandible with thin anterior edge; posterior part of process cut off from jaw. Tip of hyomandibular pouch barely touches ectoderm at level of utriculus; second and fourth do not. Cervical sinus compressed to slit; from it deep third and fourth visceral slits. Posterior pouch very long.

Bottom of laryngeal groove expanded anteriorly. Anlage of muscle around esophagus. Bronchi with ventral and lateral branches. Stomach nearly as wide as body cavity, lying on left side. Omental bursa with posterior pocket. Allantoic diverticulum extends far out into allantoic stalk. Meckel's diverticulum passes into separate anterior pouch in umbilicus.

5. Circulatory. Posterior interatrial foramen small. Pulmonary and systemic aortas separate anteriorly; pulmonary ends where it bifurcates, small artery to lung from point of bifurcation; systemic branches near thyroid as before. Caudal aorta single.

No nucleated blood cells. All blood vessels relatively smaller than in preceding stages.

6. Excretory. No open tubules. First tubules at spinal ganglion 12. Wolffian duct discontinuous in region of metanephros.
7. Endocrine. Oral portion of hypophysis with two posterior lobes, a second oral thickening below and separate from it; both free from mouth. Cells of infundibulum long; central cytoplasmic layer. Thyroid vascularized.

8. Limb buds. Fore bud with palm formed. Hind buds long and narrow, but no foot formed; mesoderm thickened.
9. Skeletal. Centra distinguishable to ganglion 33. Anlagen of cartilage bones present in upper fore limbs, visceral arches, nasal region. Loose connective tissue around brain.
10. Genital. Genital ridge becomes a large flat plate from ganglia 16 to 19, with cortical and medullary layers distinct. Milk hills are thickened ridges of ectoderm.

Age 23-16-5, left (ID) (fig. 14)

a. External.

1. Extra-embryonic. Vesicular tissue surrounding umbilical pouch.
2. Form. Balloon-shaped umbilical pouch; intestine visible through walls. Cervical flexure about 45°; prominent lumbar spine.
3. Somites. None visible externally.
4. Nervous. No parts of brain visible externally.
5. Sense organs. Oro-nasal groove obliterated; indication of eyelids; endolymphatic duct invisible.
6. Visceral arches. Gone as such; lips formed on mouth; large mandible; cervical sinus closed; broad auditory meatus; pinna and vibrissal papillae present.
7. Limb buds. Front foot rotated 60° medially; tips of toes formed on front and hind feet.
8. Circulatory. Heart eminence reduced.

b. Internal.

2. Somites. None distinguishable. Notochord with fibrous sheath into tail; practically disappears behind this. Omental bursa shows folded recesses between lobes of liver; tip cut off as mediastinum. Pleural coelom cut off laterally as far as tip of lungs (ganglion 17); pleural folds continuous with urinary ridge immediately behind. Pleural and pericardial cavities completely separate.
3. Nervous. The germinal layer of brain and cord much thinner ventrally; intermediate layer everywhere thicker, containing fiber tracts; telencephalon has intermediate layer, outer part of which contains dense layer of nuclei (two or three thick) in cortical region; olfactory lobes present. Corpus striatum partly divided anteriorly; foramen of Monro smaller; medial walls of posterior lobes of cerebrum folded out as chorioid plexi. V-shaped portion of diencephalon reduced and folded; blind pocket of third ventricle beneath corpora striata; walls of diencephalon fused dorsally (anterior commissure); fiber tract from corpora striata in walls, another running back to mesencephalon; ventricle narrow, optic chiasma below eye enlargement. Mesencephalon with reduced cavity and very thick walls; groove in ventral surface; roof forms triangle in section. Small peak in roof of metencephalon; cavity nearly as deep laterally as ventrally. No neuromeres in myelencephalon. Lateral walls of cord rounded; dorsal and ventral portions of intermediate layer distinguishable to ganglion 18; walls fused from ganglion 9 to tail; the dorsal of two cavities formed nearly obliterated.

Several layers of epidermal cells in opening of nasal pit, which opens into mouth just back of tip of jaw; dorsal expansion posteriorly in region of nerves. Eye surrounded by a groove extending two-thirds of way in; eye muscles distinct; mesenchyme and ectoderm covering lens; retinal fibers external, passing back through optic tract, where no nuclei, lumen obliterated, chorioid fissure closed. Blind duct extending from anterior corner of eye toward nasal cavity. Endolymphatic duct far from ectoderm, folded dorsally; posterior and horizontal canals complete, anterior with dorsal end only; small ventral pocket in front of cochlea, which makes one complete spiral; posterior and horizontal ampullae present; anlagen of ear ossicles present, stapes clearly defined.

Nerve I shows fibers passing to parts of nasal passage. Nerve IV present. Ganglion V differentiated into three distinct parts: medial fiber tract (mandibular), antero-lateral mixed fibers and nuclei, large posterior mass of nuclei; mandibular branch passes to muscle anlagen of jaw; maxillary divided into small parts passes to surface of lip; ophthalmic similarly in front of eye. Nerve VIII with fibers to cochlea. Ventral branch of VII passes to roof of mouth beneath hypophysis and anteriorly; posterior branch over ossicles, behind pinna to superficial posterior region of lower jaw. Upper ganglion IX closely applied to that of X. After fusion, branches of X pass back and break up dorsal and ventral to posterior end of stomach. Nerve XI relatively small, not traceable beyond first ganglion. Thirty-two dorsal root ganglia; three to twelve very close together; ganglia compact, level with bottom of cord, enclosed in vertebrae.

4. Digestive. Straight sided groove between upper lips. Tip of lower jaw narrow; mouth orifice does not extend back far. Tip of tongue free; posterior portion fits into large groove in roof of mouth; simple tooth groove in upper and lower jaws; posterior part of tongue with two grooves. Anlage of mandible in posterior part of jaw; also those of muscles, hyoid apparatus. Small bits of glandular tissue at base of tongue, lower corner of mouth cavity.

Laryngeal groove entirely closed; two flaps above glottis. No pouches remain, but vesicles in region of posterior pocket of fourth pouch, third and fourth slits; tissue glandular in appearance. Mesenchyme thickened around trachea; bronchus has one anterior, two lateral (first divided) and one ventral branch; large right ventral lobe of lung occupying median position.

Stomach greatly expanded, walls folded, curves across to right. Gall bladder in cystic lobe of liver. Gut enclosed in connective tissue and muscular layers between stomach and umbilicus. A long right posterior loop before entering umbilicus, slightly more coiled in umbilicus; long Meckel's diverticulum. Slight enlargement in perineal tubercle; entirely cut off from urinary system.

5. Circulatory. Loose connective tissue developed around the ventricles. Valves on atrio-ventricular foramina; only anterior interatrial foramen. Left atrium receives pulmonary vein. Heart about half enclosed in body wall. Pulmonary and systemic aortas separated close to heart; systemic with three valves, pulmonary with two. Right branch of pulmonary very small; right dorsal aorta blind posteriorly. Ventral aortas run through lobes of thyroid in front of systemic arch. Stapedial arteries off carotids, which eventually reach the chorioid plexi of the lateral ventricles. Vertebrals, larger than carotids, given off at systemic arches, join as basilar behind the skull, divide behind the diencephalon and join the carotids around it; small pair of posterior vertebrals. Subelavians, running backward under limb bud and down, ventral to brachial nerve, given off at same point as vertebrals; right dorsal aorta ends at this point. Left aorta becomes median about ganglion 14; small ventral artery at ganglion 22; left allantoic artery larger; right iliac given off separately.

Subelavian vein follows artery, but dorsal to brachial nerve; hyoid branch of anterior cardinal (external jugular) with branches also to side of head behind ears; extensive lymph spaces lateral to cardinals in shoulder and neck region; main branch (internal jugular) receives vein from myelencephalon near ganglion X; receives branches from rest of head near ganglion V; no large sinuses in head. Post cardinals dwindle rapidly and end at ganglion 16. At ganglion 17, behind the supra-renal bodies, the post cardinals reappear, running into the post cava on the right. Yolk sac vein receives branches from omentum and pancreatic region.

6. Excretory. Urinary ridge and tubules begin at ganglion 16; small mullerian duct in tip from ganglions 17 to 19, open posteriorly. Lumen of wolffian duct large; duct continues throughout body. Metanephros branched forward and backward from the ureters; branches arranged in semi-circle. Urethra leads to surface of perineal tubercle. Allantoic diverticulum much constricted as it leaves the umbilicus.
7. Endocrine. Oral portion of hypophysis folded posteriorly and wrapped around infundibulum, which is relatively small with very narrow opening and infolded wall, and has cavity filled with loosely packed non-epithelial cells. Anterior oral portion very dense, light staining, non-epithelial. Thyroids enlarged, extend in lobes lateral to larynx and between the two masses of glandular tissue described under no. 4. Aortic bodies from ganglia 6 to 9 are solid cords, break at aortic arch and continue, gradually reduced; by ganglion 15 largely replaced by fibers; at 16 become associated with large paired supra-renal bodies medial to urinary ridge. Aortic bodies as such (sympathetic ganglia) continue from 19. Supra-renal bodies interrupted by post cava at ganglion 20, continued as single median mass below aorta to 22. Highly vascularized; the non-sympathetic portion composed of light staining, amorphous cells.
8. Limb buds. Nerves fused. Fore bud with bones ossified as far as metacarpals, muscles differentiated in upper arm, slight grooves between four fingers. Hind bud with femur only ossified; muscles clear only in pelvic region.

9. Skeletal. Thick layer of loose connective tissue in lateral body wall outside of skeletal muscles, forming the ridge lateral to spine seen in external view, also developed around spinal cord. Following cartilage bones are ossified: centra and neural arches to ganglion 28, heads of ribs (thirteen, from vertebrae 9 to 21), scapula, humerus, radius, ulna, metacarpals, femur, nasal septum, mandible, skull floor to hypophysis.
10. Genital. Distinct gonad hanging down into coelom medial to urinary ridge from ganglion 17 to 21; in indifferent stage. Urethra runs just beneath ectoderm to end in bilobed copulatory organ, which has slight lateral folds at base. Two small nipples in front of the groin, where there is an extensive ectodermal thickening, not raised externally.
11. Muscular. Clear bundles of muscle fibers found in following places: eyes, neck, shoulder, hyoid region, upper arm, lateral to neural arches. Panniculus carnosus running from ventral ends of ribs dorsally.

Age 26-3-45, lower right (ID) (fig. 15)

a. External.

1. Extra-embryonic. Amnion attached to placenta; decidua capsularis incomplete; watery vesicles at edge of decidua basalis.
2. Form. Cervical flexure less than 45°; prominent lumbar spine; cephalic flexure relaxed.
5. Sense organs. Prominent eyelids; snout formed.
6. Visceral arches. Edge of pinna rounded and curved over, vibrissae numerous, face smooth.
7. Limb buds. Front foot rotated 90°; heel present on hand; all toes clearly marked off.
8. Circulatory. No external heart elevation.

b. Internal.

2. Somites, etc. Within the centra the notochord shrunken; between them expanded, with cells concentrically arranged around it. Posterior coelom large, contains coiled gut; bladder projects out into coelom; wolffian ducts suspended by mesentery; all mesenteries narrow; lobes of liver and fold of omentum as far back as umbilicus, the mouth of which is wide; pleural and peritoneal cavities separated by muscular diaphragm; post cava projects into right pleural cavity; wall of pericardium free except ventrally.
3. Nervous. Germinal layer thin throughout, slightly thicker dorsally; dense layer of cortex is thick; intermediate layer everywhere thickened and fiber tracts more prominent; marginal (fibrous) layer of cord thick. Olfactory lobe with anterior blind pocket; foramen of Monro small, chorioid plexus extending out through the closed portion. V-shaped portion of diencephalon relatively small; pineal body present as button on roof. Cavity of mesencephalon reduced to a slit. Lateral pits in cavity of metencephalon. Thin roof and side walls of metencephalon and myelencephalon formed into chorioid plexus; the myelencephalon strongly folded against the metencephalon. Dorsal portion of cord wider than ventral; deep ventral groove; single cavity; intermediate layer with dorsal and ventral horns; fibrous layer interrupted dorsally.

Nasal openings closed by epidermal tissue; anterior part of cavity crescent shaped, with ectoderm folded into small pits and vesicles; posteriorly a central island (attached posteriorly) in the enlarged cavity; enters mouth behind tip of tongue. Lids and lens of eye enlarged, the latter occupying about half the cavity; retina with intermediate layer; tear gland replaces duct at anterior corner of eye. Endolymphatic duct enclosed in skull; rest of ear in bulla; canals long and complete; pinna folded; cochlea makes one and two-thirds turns; ossicles ossified, the incus and malleus much larger than the stapes, the malleus in contact with the cartilage bone of lower jaw.

Nerve III emerges from skull at level of pituitary and runs close to ganglion V, which is continued forward in the skull. Upper ganglia of IX and X in contact with large cervical sympathetic ganglion behind bulla; at the point where XII separates X gives branches to larynx and sympathetic.

4. Digestive. Lip groove narrow; anlagen of dorsal incisors present; lips inclined medially; anlagen of ventral incisors shaped like fleur-de-lis in section, surrounded with epidermal tissue. Tooth groove internally expanded posteriorly. Dermal calcification external to cartilage bone of lower jaw. Tongue muscles well developed; no posterior grooves. No pouches remaining except eustachian tube. Tissue derived from visceral slits enlarged, shows many mitoses. Pharynx forms diamond-shaped opening above glottis. Lungs much enlarged, bronchi extensively branched, bulk still smaller than heart; lung extends from ganglion 14 to 19. Phrenic nerve passes to diaphragm. Esophagus with connective tissue and muscular layers; this continued to point where gut emerges from umbilicus. Cavity of stomach round; epithelium thickened and folded in pyloric region; lobe anterior to entrance of esophagus. Gut with further coils before entering and inside umbilical pouch; also coiled posterior to pouch; Meckel's diverticulum long, running out of umbilicus and posteriorly in coelom.
5. Circulatory. All vessels relatively small. Auricles divided into cells by septae; right atrioventricular foramen with one valve flap, left with two; interventricular foramen narrow; systemic and pulmonary aortas separate to heart.

No right dorsal aorta and no right branch of pulmonary, or systemic. Three branches on the left from the top of the arch, dorsal (left vertebral) and ventral (right innominate) larger than the central (left innominate). Subelavians (external to ribs) and internal mammary (internal) run back from innominates. Internal carotid runs as far as ganglion V, and vestige left at the brain, but no apparent connection. Basilar artery with large branches to ear; surrounded by the dienecephalon and metencephalon where they touch. Segmental arteries posterior to diaphragm with single dorsal roots. Small artery to dorsal side of stomach in front of pancreatic; extensive branches to mesenteries from yolk sac artery; paired lateral arteries to supra-renals at ganglion 20; ventral pair in front of single portion of supra-renal at ganglion 21; allantoics same size, iliaes off base; large external iliac to limb and internal iliac to perineal region.

- Pulmonary vein with two anterior and two posterior branches. Internal jugular enters skull behind and beneath bulla. Post cava very large in front of liver; only remnant of left post cardinal behind junction with post cava; allantoies can now enter liver almost directly from umbilicus; iliaes run some distance posteriorly and enter leg.
6. Excretory. Urinary ridge begins at ganglion 19, just back of the diaphragm. Open mullerian and wolffian ducts extending nearly the whole length; mullerian duct ends blindly. Metanephros with extensively branched cavities, arranged in circle; the ureters enter the base of the bladder, separate from the wolffian ducts which join the upper end of the urethra; metanephric tubules arranged in pyramids.
 7. Endocrine. Anterior oral portion of pituitary enlarged and closely applied to the oral vesicle. Thyroid very vascular; divided into right and left separate portions. Suprarenal bodies highly vascular, about as large as metanephroi; anteriorly enclosed in compact mesoderm.
 8. Limb buds. Fore limb free from body at elbow. All bones present and ossified except two distal phalanges. All important muscles clearly outlined and anlagen of tendons present. No marginal vein or ectodermal thickening on fore foot. Hind limb ossified to same extent; no tendons; marginal vein and ectodermal thickenings persist.
 9. Skeletal. Layer of loose connective tissue much thicker than preceding stage; especially well developed between skeletal and skin muscles and in the region of the shoulder hump, being dorsal to the nerve cord in this region. Similar tissue developed below the ribs above the lungs, and nervous system enclosed in an extensive layer of it. Vertebrae ossified into tail; neural arches enclose about half of cord; all ribs ossified except tips of free ribs. First five ribs connected with slightly ossified anlagen of sternbrae, which gradually separate posteriorly. Skull floor bones, occipitals, hyoid and larynx, bulla, and limb bones ossified. Odontoid process of axis a separate element. Dermal bones, showing strikingly different type of ossification, ossified as follows: dentary, maxilla, premaxilla, frontal, parietal, clavicle.
 10. Genital. Gonad a large lobe with narrow mesentery; anterior tubules associated with it; testis cords appear to be differentiated in this specimen. Penis with glands developed, heavy folds at base. No indication of accessory glands. Mammary gland a solid spherical mass beneath ectoderm; indications of differentiated core; not developed beneath accessory nipples.
 11. Muscular. All important skeletal muscles appear to be differentiated as distinct bundles of fibers without sheaths or striations.

SUMMARY

1. It was found that in the guinea pig caesarian section with ether anesthesia produced vaginal smears indicative of oestrus at 4 to 5 days thereafter, as contrasted with almost immediate oestrus following parturition.

2. A new measure for the state of development of mammalian embryos is described: the earliest age at which a particular developmental event takes place.

3. A brief general description of guinea pig development is set forth.

4. A 'Normentafel' for the guinea pig, based upon the above measure and running up to 26 days after copulation, has been compiled.

5. Directions for the use of the table are given.

6. It is concluded that externally the visceral arches and internally the circulatory system show the most satisfactory series of definite morphological changes correlated with age. Histological changes are unsatisfactory because of their gradual nature.

7. It is concluded that the new measure is a more satisfactory one than size, somite number, or average state of development.

LITERATURE CITED

- BISCHOFF, T. L. W. 1852 *Entwicklungsgeschichte des Meerschweinchens*. J. Ricker, Giessen.
- DRAPER, R. L. 1920 The prenatal growth of the guinea pig. *Anat. Rec.*, vol. 18, pp. 369-392.
- HARMAN, M. T. AND M. PRICKETT 1932 The development of the external form of the guinea pig (*Cavia cobaya*) between the ages of 11 days and 20 days of gestation. *Am. J. Anat.*, vol. 49, pp. 351-378.
- 1933 The development of the external form of the guinea pig (*Cavia cobaya*) between the ages of 21 days and 35 days of gestation. *J. Morph.*, vol. 54, pp. 493-519.
- HENSEN, V. 1876 Beobachtungen über die Befruchtung und Entwicklung des Kaninchens und Meerschweinchens. *Zeitsch. f. Anat. u. Entwickl.*, Bd. 1, S. 213-273, 353-423.
- HUBER, G. C. 1918 On the anlage and morphogenesis of the chorda dorsalis in mammalia, in particular the guinea pig (*Cavia cobaya*). *Anat. Rec.*, vol. 14, pp. 217-264.

- IBSEN, H. L. 1928 Prenatal growth in guinea pigs, with special reference to environmental factors affecting weight at birth. *J. Exp. Zool.*, vol. 51, pp. 51-94.
- LAMS, H. 1913 Étude de l'oeuf de cobaye aux premiers stades de l'embryogenese. *Arch. de Biol.*, T. 28, pp. 229-323.
- LIEBERKÜHN, N. 1882 Über die Chorda bei Säugethieren. *Arch. f. Anat. u. Entwickl.*, S. 399-438.
- 1884 Fortsetzung. *Ibid.*, S. 435-452.
- LOUTTIT, C. K. 1927 Reproductive behaviour of the guinea pig. I. The normal mating behaviour. *J. Comp. Psych.*, vol. 7, pp. 247-263.
- MACLAREN, N. 1926 Development of Cavia: Implantation. *Trans. Roy. Soc. Edinburgh.*, vol. 55, pp. 115-123.
- MACLAREN, N. AND T. H. BRYCE 1934 The early stages in the development of Cavia. *Trans. Roy. Soc. Edinburgh*, vol. 57, pp. 647-664.
- MALL, F. P. 1918 On the age of human embryos. *Am. J. Anat.*, vol. 23, pp. 397-422.
- NELSON, W. O. 1933 Reciprocal relationship between ovaries and anterior hypophysis as factor in control of lactation. *Proc. Soc. Exp. Biol. and Med.*, vol. 30, pp. 953-954.
- SANSOM, G. S. AND J. P. HILL 1931 Observations on the structure and mode of implantation of the blastocyst of Cavia. *Trans. Zool. Soc. London*, vol. 21, pp. 295-346.
- SELENKA, E. 1884 Studien über Entwicklungsgeschichte der Thiere, Bd. 3. Wiesbaden.
- SPEE, F. GRAF 1901 Die Implantation des Meerschweincheneies in die Uteruswand. *Zeitsch. f. Morph. u. Anthropol.*, Bd. 3, S. 130-182.
- SQUIER, R. R. 1932 The living egg and early stages of its development in the guinea pig. *Carnegie Institution Contributions to Embryology.*, vol. 137, pp. 225-250.
- WRIGHT, S. 1935 A mutation of the guinea pig, tending to restore the pentadactyl foot when heterozygous producing a monster when homozygous. *Genetics*, vol. 20, pp. 84-107.
- YOUNG, W. C., E. W. DEMPSEY AND H. I. MYERS 1933 Some data from a correlated anatomical, physiological and behaviouristic study of the reproductive cycle in the female guinea pig. *Am. J. Physiol.*, vol. 105, pp. 393-398.
- 1935 Cyclic reproductive behaviour in the female guinea pig. *J. Comp. Psych.*, vol. 19, pp. 313-335.

THE EMBRYOLOGY OF THE GUINEA PIG

II. THE POLYDACTYLOUS MONSTER. A NEW TERAS PRODUCED BY THE GENES $PxPx$ ¹

J. P. SCOTT

Whitman Laboratory, The University of Chicago

ONE TEXT FIGURE AND THREE PLATES (TWENTY-TWO FIGURES)

AUTHOR'S ABSTRACT

A detailed study of Wright's polydactylous monster (produced by a semi-dominant lethal gene) indicates that it belongs to a general type found also in rare human cases. The diagnostic characteristics are: clubbed feet and approximately double the usual number of digits, embryonic posture, microphthalmia and enlargement of the diencephalon, and missing tibia and telescoped sternum; all organ systems in the body except the genital and circulatory are grossly abnormal. The defects appear to be produced by an arrest of morphogenesis and an alteration of relative growth rates. It is indicated that a controlling center of digit formation exists on the lateral (postaxial) side of the foot, that skeletal and dermal structures are controlled by it, but that muscles are differentiated according to the area of the limb in which they lie. The gene itself is not atavistic, although its effects in the heterozygote have that appearance.

CONTENTS

Introduction	299
Materials and methods	300
The anatomy of newborn monsters	301
The anatomy of heterozygous polydactyls	307
Discussion	309
Summary and conclusions	313

INTRODUCTION

A new hereditary anomaly of the guinea pig has recently been described by Wright ('34, '34a and '35). Guinea pigs usually have four toes on the front foot and three on the hind foot. Those heterozygous for the gene Px have a tendency toward the restoration of lost digits, especially the pollex but also the first and fifth toes of the hind foot. Breeding results indicate that the controlling factor is semi-dominant and lethal when homozygous.

¹The material embodied in this paper forms part II of a doctoral thesis in zoology submitted to the faculty of The University of Chicago.

From matings between heterozygous guinea pigs Wright obtained a few dead monstrous embryos (presumably the missing homozygotes) as well as a few monsters which died at birth. These extraordinary animals were marked at all ages by the following external defects: paddle-shaped feet bearing from nine to twelve very short toes and clubbed so that the palmar and plantar surfaces were directed toward instead of away from the body, bulging and expanded heads, and microphthalmia. Certain other defects were occasionally present: protruding brain in the parietal region, and harelip.

Further study indicates that the newborn monster belongs to a type almost unique in teratological literature. The condition of the feet throws new light on the mechanism of embryonic differentiation of the digits, and an interesting question concerning the physiological properties of the gene *Px* is raised by the fact that its effects in the heterozygote appear to be in part atavistic. In addition, the following description of the detailed anatomy of the newborn homozygotes and heterozygotes has significance as a basis for the study of the embryological effects of the gene.²

MATERIALS AND METHODS

Nine newborn *PxPx* monsters were available for study, together with many more newborn and adult heterozygotes. The numbers obtained indicate that in the parent stocks (I and ID) about one homozygote in twelve survives until the time of birth.

The methods used were chiefly those of gross dissection, although histological examinations were made of the bones, intestine and spinal cord. A Spalteholz preparation was made of each of the three genetic types (fig. 1); the description of minor skeletal features is based on this material. The small available number of newborn monsters made possible only a very rough description of the amount of variation in the homozygote. It is probable that such variation will be found to be

² Most of the monsters die at the beginning of the fetal period. Their development up to this time is described in another paper (Scott, '37 a).

large when *PxPx* animals have been recovered from other hereditary stocks, as this should provide opportunity for interaction with new factors.



Fig. 1 Spalteholz preparations of newborn guinea pigs with 41, 21 and 14 toes, respectively. (*PxPx*, *Pxpx*, and *pxpx*.) $\times \frac{3}{4}$.

THE ANATOMY OF NEWBORN MONSTERS

The study of this series of monsters has brought many new abnormalities to light, and Wright's general external description has been confirmed. A condensed list of abnormalities found in various parts of the body is given below.

A. External Anatomy

1. Posture. Vertebral column flexed in C-shape; abdomen flat.
2. Proportions (see table 1). Skull width and depth and foot width increased; total length and upper arm length normal; snout length and limb measurements other than above decreased.
3. Hair. Short in some as in premature birth.
4. Head. Bilateral harelip, roofless mouth, and short mandible. Skull enlarged with soft spot in parietal region; or of normal size with protrusion of brain in same region; always deformed. Only fused lids of eye present; largest 3.5 mm.

5. Genitalia. Nipples missing in about half of cases.

6. Limbs (figs. 2, 8).

a. General. Feet clubbed, digits multiplied and fused. Toes in three groups, lateral group resembling normal digits III-V but strongly flexed. Central group usually with four small fused toes weakly flexed; medial group usually with two straight toes and one free weak medial toe.

TABLE 1¹

Body measurements of newborn guinea pigs in centimeters

MEASUREMENT	MEAN			DIFFERENCE S.E. OF DIFFERENCE	
	50 living <i>pxpx</i> (ID)	21 living <i>Pxpx</i> (ID)	6 dead <i>PxPx</i> (mixed stock)	<i>pxpx-Pxpx</i>	—
Tip of nose to tip of tail	11.742±0.171	12.297±0.232	11.027±0.416	—1.914	1.599
Head length	4.246±0.033	4.320±0.049	3.793±0.235	—1.294	3.549
Head width (zygomatic arch)	2.049±0.020	2.093±0.025	2.320±0.092	—1.390	—3.590
Head depth, in- cluding jaw	2.231±0.026	2.295±0.031	2.462±0.123	—1.562	—2.670
Tail-knee length	3.312±0.045	3.324±0.081	2.560±0.150	—0.143	4.335
Knee-heel length	3.795±0.042	3.783±0.053	2.312±0.113	0.187	5.456
Middle hind toe to knuckle	0.720±0.012	0.686±0.015	0.615±0.035	1.730	2.914
Shoulder-elbow length	2.480±0.030	2.561±0.050	2.464±0.099	—1.462	0.198
Elbow-wrist length	2.960±0.034	3.057±0.044	2.280±0.134	—1.717	4.488
Middle front toe to knuckle	0.570±0.012	0.531±0.010	0.402±0.015	2.344	4.384

¹ Statistics in this table computed and checked by Dr. W. L. Russell.

b. Front. Foot flexed ventrally, or rarely whole limb straight and foot flexed on radius (unilateral condition only). Outermost toe usually bears small nubbin. Distal metacarpal pad repeated at base of central group; heel pad divided lengthwise.

c. Hind. Foot strongly flexed on the tibia. Outer basal metatarsal pad (not present on normal) restored; distal repeated opposite central group of toes; medial pad sometimes doubled. Plantar sesamoid ('heel') pad shifted distally and laterally.³

³ The metatarsal foot pads of rodents are typically arranged in a triangle at the bases of digits II, III-IV, and V, with a small fourth pad at the base of digit I.

- B. Skin. Excessive amounts of subcutaneous fat present.
- C. Nervous system.
1. Peripheral.
 - a. Cranial nerves. Optic absent.
 - b. Sense organs. Usually small orbit with no eye, muscles inserted on lacrimal gland which is attached to vestigial lids; or reduced eyeball buried in orbit; sometimes eye reduced asymmetrically. Nose with harelip, cleft palate and reduced upper lip, or normal.
 2. Central. Brain with first three ventricles fused into common cavity which has no connection with swollen aqueduct and which is filled with blood; either hydrocephalous or with tissue protruding from between hemispheres. Halves of cerebrum separate, corpus callosum a ridge on either side, olfactory lobes rotated to side. Diencephalon greatly enlarged, touches roof of skull, where tissue thickened; walls entirely separate; pineal body close to skull and sagittal veins reduced. Mesencephalon similar to that of late embryo with no colliculi; slightly asymmetrical and touches skull. cerebellum asymmetrical, overlaid by mesencephalon; no vermis or pons. Medulla enlarged; fold where basilar artery enters brain upright instead of inclined forward (figs. 20, 22).
- D. Digestive system. Tube reduced in length and diameter from esophagus to to colon; abdominal cavity not filled. Liver compressed; pancreas reduced proportionately to duodenal loop. Walls of intestine thick, caused by thickening of peritoneal layer.
- E. Respiratory system. Lungs greatly reduced, as are pleural cavities which lie entirely dorsal to heart. Trachea, central tendon of diaphragm reduced.
- F. Circulatory system. No defects except those directly associated with abnormal parts.
- G. Excretory system. Kidney lobulated externally (fetus-like), bladder lengthened to reach forward position of umbilicus.
- H. Endocrine system. No gross abnormalities discovered.
- I. Skeletal system.
1. Skull (figs. 14 to 16). Mandible abnormally curved, diastema shortened, incisors do not meet. In some cases maxillae and palatines do not fuse in mid line, the premaxilla being divided by the orno-nasal opening. Occipital region swollen; lambdoidal ridge rounded. Zygomatic arch pushed dorsally proportionately to reduction of eye. Roofing bones of skull expanded according to expansion of brain, leaving opening in center of skull; interparietal unusually large.
 2. Axial (figs. 1, 17). Vertebrae shortened; neural arches flattened, in one specimen not fused in lumbar region; caudals normal. Ribs reduced in size and length; close together. Sternum shortened; sternbrae fused, the fourth pair enclosing the third.

3. Limbs (figs. 5, 11).

- a. Fore. Acromion process of scapula forms obtuse instead of right angle. Clavicle shortened and thickened. Humerus, radius and ulna altered in length (see table 1). Pisiform and radial sesamoid separated in divided heel pad. Carpals of distal row multiplied, misshapen. Seven or eight metacarpals, lateral three largest, medial and central often fused basally. Basal phalanx shortened and usually doubled; middle reduced; in central and medial groups often two toes on a single metacarpal; or on medial side free.⁴
- b. Hind. Blade of ilium broad; pubic and ischial symphyses unossified. Lengths of femur and fibula altered; medial condyle of femur reduced, patella missing; tibia missing except for parts of epiphyses; fibula stout, curved, proximal end enlarged. Tarsal four expanded, lies above 2 metatarsals; tarsals one and two multiplied, irregular. Tibial sesamoid missing; plantar sesamoid reduced to splint under 'heel' pad. Five metatarsals; inner two branched, with extra ones inserted between branches. Middle phalanx reduced. Often two toes on single metacarpal in central groups.⁵

J. Genital system. No gross abnormalities of either sex.

K. Muscular system.

1. General. All stringy in texture and difficult to separate, more or less abnormal in shape, some fusion.
2. Limbs.
 - a. Fore. All abnormal in shape. Abnormal insertions: clavo- and acromio-trapezii rolled under edge of metaacromion process, spino-trapezius on edge of latter; pectoral muscles rolled under anterior end of sternum at origin and xiphi-humeralis inserts external to deep pectoralis; biceps brachii on median epicondyle of humerus and pronator teres. Extensor pollicis on second metacarpal instead of I; e. digiti quinti on three lateral toes instead of two; e. digitorum communis to all toes but principally central and medial groups; e. carpi radialis to third metacarpal from outside and second and third from inside, instead of digits II and III. Flexor digitorum profundus with enlarged tendon, to all toes except most medial; f. digitorum sublimis principally to central group instead of digits II-IV; palmaris to ligaments of wrist instead of heel pad.

⁴In the adult guinea pig there may be as many as eighteen small bones in the carpus, but in the newborn animal only ten are ossified. Proximal row: pisiform, ulnar, radial and radial sesamoid (radial accessory). Distal row: fused carpals 4 and 5, carpal 3, central, minute carpals 1 and 2, and vestige of metacarpal I. The pisiform and radial sesamoid meet in the heel pad (fig. 6).

⁵The following bones are found in the normal tarsus at birth. Proximal row: fibular and tibial. Distal row: tarsal 4 (probably fused 4 and 5), tarsals 3, 2 and 1. The central lies between the rows, above tarsals 2 and 3. Medial to it and above tarsal 1 is the tibial sesamoid. On the plantar surface is the elongate plantar sesamoid, lying between the tibial sesamoid and tarsal 1 on one side and the fibular on the other and supporting the 'heel' pad. A vestige of metatarsal V is present (fig. 12).

- b. Hind. Muscles fused so as to be practically immovable, especially in gluteal region; femur abducted and extended at right angles to sagittal plane. Abnormal insertions: gracilis, semi-membranosus, biceps femoris on fascia over shank instead of tibia; tibialis on ankle ligaments instead of tarsal 1; extensor digitorum longus to all toes in three groups, instead of three; flexor digitorum communis to all except most medial toe.

The fundamental abnormalities of the animals described above appear to be: 1) Doubling of the usual number of digits, or nearly so, and clubbed feet, 2) embryonic posture, 3) microphthalmia and enlargement of the diencephalon, and 4) missing tibia and telescoped sternum. Other abnormalities are of course present but are more variable and not so easily recognized. Taken together the above may be considered diagnostic of a hitherto undescribed type of congenital malformation. The name, 'polydactylous monster,' is apt and is correct in that ordinary cases of polydactyly are not sufficiently aberrant to be called monsters.

The limbs are about two-thirds as long as the normal, but the feet are about twice as wide. The general effect appears then to be more a change in proportion than a change in size. The clubbing of the hind foot may be attributed to the pull of shank muscles unopposed by the tibia, but there is no such condition of the radius in the front foot. There is a high degree of correlation between the development of the bones of the foot and such external structures as pads and claws; higher, in fact, than between certain skeletal elements such as phalanges and metacarpals. Both sets of structures indicate that the lateral side of the foot is fairly normal, but that the medial and central groups of toes may represent multiplied digits I and II. On the other hand, there is a poor correlation between the development of bones and tendons. A muscle normally inserted on digits III and IV of the front foot will pass to the central group of toes rather than to those of the lateral group having the appearance of III and IV. The muscles appear to pass to the correct region of the foot rather than to the correct bone. Numerous abnormal insertions in other parts of the limbs support this view. The

general explanation appears to be this: the differentiating area controlling the shape of the foot is located laterally, and because of the size of the foot the medial region has escaped control, resulting in doubling of digits. Bones and dermal structures are laid down according to the shape of foot so produced. But muscles are laid down according to the region of the foot rather than its shape, tending to insert on every bone in that region.

The bent head, C-shaped flexure of the back and even the position of the feet are similar to those of the 26-day embryo figured in the previous paper (Scott, '37). In addition, the brain more closely resembles that of a 30-day fetus than a normal newborn animal, and the shape of the kidney resembles the fetal type. It is difficult to avoid the conclusion that an arrest of morphogenesis has taken place, the effect being more severe on the nervous system (which determines the flexure of the embryo), limbs and kidneys than on other organs. Such an arrest might also explain the harelippled condition. On the other hand, the only indications of histological defects discovered were the abnormal distribution of fat and the structure of the intestine; there were no cases of undifferentiated tissue.

The defects of brain and head are for the most part well correlated and require no new type of explanation. The shape of the skull roofing bones corresponds to the shape of the brain, and the position of the zygomatic arch to the size of the eye. Reference has already been made to the fetal and embryonic characteristics of the brain and upper jaw. But the diencephalon is relatively much larger than in the fetal condition, indicating an alteration of relative growth rates. The eye has suffered the most striking reduction, but the length of the intestine is also affected. Alteration of growth rates might also produce the stoppage of the aqueduct and resulting hydrocephaly.

Finally, there appears to be no obvious explanation for the missing tibia, nor for the occasional non-appearance of the nipples. The telescoped sternum is of course correlated with

the reversed flexure of the thorax which leaves no room for the former in an extended condition.

Most of the abnormalities are thus explainable as the result of an arrest in morphogenesis plus an alteration of growth rates. Since morphogenesis is the result of differential growth the two are probably directly connected. Muscular anomalies are probably secondary, produced by lack of correlation in position between somites and other organs resulting from action of the first two factors.

Of the different organ systems only the circulatory and genital appear to be unaffected. The genital system is one of the last to differentiate and perhaps missed the critical period. The circulatory system is differentiated by the pressure put upon it and is not directly or indirectly affected by morphogenetic processes in its major parts. As a matter of fact, the minor branches are abnormal, since there must be a blood supply to all the toes.

The lungs in none of the monsters were inflated, and it is probable that malformations of the chest and its musculature make it impossible for them to breathe. Also, the brain is very likely to be injured during birth.

THE ANATOMY OF HETEROZYGOUS POLYDACTYLS

Wright ('35) has described the *Pxpx* animals as having the following characteristics: 1) a tendency toward the restoration of the thumb, little toe, and big toe, in order of magnitude of effect, and the very rare addition of a sixth finger, 2) an occasional tendency toward clubbing of the foot, 3) an average increase of 7% in birth weight over homozygous normals, and 4) an increased mortality in a certain cross. Since then one animal with a vestigial sixth digit at the base of the thumb has been discovered. A list of abnormalities discovered by measurement and dissection follows.

A. External anatomy.

1. Posture. Appear to be stockier (extremities thickened).
2. Proportions (see table 1). Front toe shortened.
5. Genitalia. Several with extra nipples (also in normal animals).
6. Limbs (figs. 3, 9).
 - a. General. Extra digits similar to corresponding ones in other rodents, vary greatly in state of development, but claw and distal phalanx always present. Sensation present, but can be moved only slightly.
 - b. Fore. Thumb typically with small claw and one or two phalanges, loosely attached. New pad at base sometimes present.
 - c. Hind. Big toe a short stub. Little toe normal in appearance, set farther back than rest; outer basal metatarsal pad occasionally present.

I. Skeletal system.

2. Axial (fig. 18). Third and fourth pairs of sternebrae fail to fuse, producing hole through sternum in adult.
3. Limbs (figs. 6, 12).
 - a. Fore. Metacarpal I enlarged, two short phalanges do not articulate with it; central enlarged. Splint in extra digit at base of thumb (one case).
 - b. Hind. Metatarsal V restored, no tarsal. Metatarsal I, with elongate basal and small distal phalanx may be restored. Tarsal 1 misshapen, tarsal 2 enlarged and forced to medial side, tarsal 3 greatly enlarged.

K. Muscular system.

2. Limbs.
 - a. Fore. Thumb usually connected only with median nerve and palmar tendon; slight trace of flexor pollicis restored (one case).
 - b. Hind. Fifth hind toe connected with nerves, plantar tendon, and branch of peroneus quartus. Hallux innervated, slight attachment to plantar tendon.

The defects of the heterozygote appear to be of the same nature as those of the monsters but considerably less than half as severe. The small result of the heterozygous condition might be accounted for as either a time effect or a threshold effect. No new abnormalities appear in the adult heterozygote and, as Wright ('35) showed, the difference in weight does not persist. It therefore seems likely that the gene *Px* acts only in the prenatal period.

The restored thumb, big toe and little toe are probably homologous with the lost ancestral digits, the two phalanges of the thumb and big toe indicating that there is no repetition of adjacent digits. But the restoration of the bones and dermal structures has not brought back the missing muscle

(peroneus quintus), and all muscle normally connected with the basal elements of the restored digits are unaffected in the heterozygote. This supports the previous conclusion that the position of muscles is not determined by shape and further suggests that the gene *Px* is not entirely similar in action to the gene or genes which produced the ancestral digits.

The condition of the skeleton in the heterozygous animals demonstrates clearly that the bone which Harman and Saffry ('34) identified as the phalanx of the thumb is really the radial accessory, since both phalanges of the thumb, *and* the radial accessory appear when the thumb is restored. Furthermore, figure 4 given by these authors shows the bone to be proximal to metacarpal I, in the position usually occupied by the rodent radial accessory. Otherwise their account is confirmed.

DISCUSSION

Polydactyly may be defined as a condition in which supernumerary digits form a more or less regular addition to the normal series. It is therefore distinct from the 'hyperdactyly' so often described in connection with amphibian regeneration and transplantation experiments, in which reduplication of the limbs is involved. On the other hand, it may be related to the condition found in certain ancient Amphibia which normally had more than five digits.

Polydactyly is found both in animals with the full five digits (mouse, man) and in those which normally show some reduction of the digits (cat, dog, hen, guinea pig, horse). The latter category has attracted considerable attention on account of its evolutionary significance (Stockard, '30). Judging from Bateson's (1894) attempt to collect all available instances, both forms of the anomaly are rare among wild animals. A six- or seven-toed condition appears to be fairly common among the cats in this country, the author having noticed three cases in the course of a year, and according to Stockard certain breeds of other domestic animals are always polydactylous.

All these cases of true polydactyly are essentially similar in that the bones above the carpus and tarsus are not multiplied, and in all the size and completeness of the extra digits vary a great deal. The polydactylous mouse of Bagg ('29) departs from the usual type in showing gross malformations of the foot and fusion in the carpus and tarsus (Bean, '29). The case of the cat is also unusual (Bateson, 1894), in that there is a tendency for the medial digits to show reversed symmetry of the claw. This is not true in the guinea pig, which does not have a retractile claw. In fact, guinea pig polydactyly in all its forms is structurally similar to the usual type, the feet of the monsters being only an extreme expression.

However, the entire complex of abnormalities found in the polydactylous monster of the guinea pig has few counterparts in the literature. The 'luxated' trait of mice (Hovelaque, '20) shows missing tibia, clubbed hind feet and occasional polydactyly, indicating, if taken with the guinea pig case, that these three traits are causally connected. But the other abnormalities of the polydactylous monster are missing, and the present author has been unable to find anything in zoological literature which resembles it.

Human teratology has been more extensively reported, and Broman ('11) has figured a human monster with six digits on all four extremities, a slight tendency toward clubbing of the feet, harelip on the left side, and a large protrusion of the brain in the neck region. In a private communication Doctor Broman states that the penis is missing, the right testis undescended, the eyes of normal size, and the left fifth toenail doubled. The father was unknown, but both the mother and grandmother were reported to be normal. The resemblance of this monster to the guinea pig type appears to be superficial, especially since extra digits are fairly common on human terata.

Davidson ('18) has described an otherwise normal 2-year-old child as having enlarged hands slightly flexed on the radius, five fingers and no thumbs, missing tibia, short fibula, clubbed feet, eight toes on each foot (preaxial polydactyly), and the

innermost toe only slightly ossified. There was no record of abnormality among the relatives. This is probably a low grade of polydactylous monster produced by environmental causes.

Pilmanis ('33) has described a 4½-month fetus with thirty-four digits, harelip, cleft palate, clubbed feet, syndactyly, uncalcified tibia, and bowed fibula. He ascribes the origin of the defect to the germ plasm. Further details are unavailable, as the original paper cannot be obtained in this country, but the abnormalities listed clearly indicate that this monster belongs to the type found in the guinea pig.

It is reasonable to conclude that the polydactylous monster belongs to a hitherto unrecognized and very rare general type. This brings up the possibility of its appearance in man as the result of a mating between polydactylous parents.

Inherited cases of polydactyly may be controlled either by multiple factors, as in the little-toe type of polydactyly first described by Castle ('06) and in the polydactyly of the hen (Punnett and Pease, '29), or by unit factors, as in the mouse, guinea pig, and probably man and the dog. In the guinea pig the unit factor appears to affect the medial (preaxial) side of the limb primarily, whereas the multiple factors almost always affect the lateral (postaxial) side of the limb only. This distinction cannot be carried over into other forms. In the hen polydactyly is produced by division of the hallux. In man postaxial polydactyly has been described as produced by a dominant factor (Sverdrup, '22) and by a recessive factor (Snyder, '29). Both preaxial and postaxial polydactyly, or either, a dominant condition, occur in one of the cases described by Pftzner (1898).

Although it is probable that several different kinds of hereditary factors produce polydactyly in various mammals it is still possible that a human gene similar to the guinea pig *Px* exists and that the homozygote has remained undiscovered because of the rarity of matings between polydactylous persons and the probable early fetal death of the monsters.

The variety of genes producing polydactyly does not suggest that they are latent heredity derived from a common amphibian ancestor. And it is obvious that the gene Px is not atavistic; otherwise, some very peculiar fossil guinea pigs remain to be discovered. It would appear that the factor is a general one which increases the number of digits, and only the fact that some are missing in the guinea pig causes it to appear atavistic. Nevertheless, the presence of considerable latent heredity, probably present in greater quantity in some individuals than others, is evidenced by the occasional production of fairly normal extra digits in a heterozygote. It is not unreasonable to suppose that the factors concerned in multiple factor restoration of the little toe are such latent heredity and hence atavistic.

The operation of the $PxPx$ genes does give some idea of what factors would be involved in complete atavistic restoration of the digits. As was earlier pointed out, the development of the skeleton and dermal structures of the foot is correlated with external form, whereas that of the muscles corresponds to the region of the foot irrespective of its form. Nerves and blood vessels were studied in a case of preaxial polydactyly in the cat (Scott, Wedding and Kirtley, unpublished). The condition of bones and tendons was similar to that in the guinea pig, but the nerves and blood vessels, which were closely connected, simply supplied all parts of the foot in an irregular fashion, irrespective of the homologies indicated. Thus the restoration of a primitive foot would require the action of factors 1) restoring the form of the foot, and 2) correlating the development of muscles with that of the skeleton. Restoration of nerves and blood vessels would be accomplished by functional adaptation.

Although most of the abnormalities can be explained as the direct or indirect results of an arrest in morphogenesis and an alteration of relative growth rates, some challenging exceptions remain. One is the full size of the optic chiasma, ordinarily supposed to be dependent on the development of the eye. Another is the occasional loss of the nipples in homozygotes although heterozygotes often have extra ones as do

the normal animals of these stocks. A third is the missing tibia and unaffected (except as it is somewhat strengthened and bent) fibula to which it is normally adjacent.

SUMMARY AND CONCLUSIONS

1. The paper is a study of the effects of Wright's guinea pig gene *Px*, a semi-dominant lethal whose outstanding effect is the production of extra digits, especially the pollex.

2. The results are based on dissections and anatomical preparations of nine newborn homozygous animals (monsters of the type briefly described by Wright) and numerous heterozygotes and normals of various ages.

3. The most prominent abnormalities of the monsters are: clubbed feet and approximately double the usual number of digits, embryonic posture, microphthalmia and enlargement of the diencephalon, and missing tibia and telescoped sternum.

4. Other defects are: embryonic form of brain and kidney, reduction of digestive and respiratory systems, deformation of skeleton correlated with that of associated organs, and fusion and abnormal attachments of the muscles.

5. Monsters may occasionally show hydrocephaly, harelip, and missing nipples.

6. It is concluded that the polydactylous monster is a distinct new type in teratology, known elsewhere only through extremely rare human cases.

7. The heterozygotes show (besides extra digits and increased birth weight) shortened front toes, altered ankle and wrist bones, and a hole through the sternum.

8. It is concluded that the effects of the gene in the heterozygote are far less than half those in the homozygote; this may be either a threshold or a time effect.

9. The most general effects of the gene appear to be an arrest of morphogenesis and an alteration of relative growth rates.

10. Development of dermal and skeletal structures appears to be correlated with form and that of muscles with position, while that of nerves and blood vessels can be accounted for

as the result of physiological adaptation. Defects in these organs are probably secondary effects of the gene.

11. The polydactylous foot appears to be produced by interference with the controlling center of digit formation, located on the lateral (postaxial) side.

12. The lethal effect of the gene is remote, apparently caused by abnormal musculature which prevents breathing.

13. The 'atavistic' effect of the gene is accidental, made possible by the presence of enough truly atavistic heredity to make extra digits appear natural. It is indicated that factors correlating the development of muscles and bones are missing in the normal guinea pig, as well as those increasing the number of digits.

14. The phalanges of the pollex are not found in the normal newborn guinea pig.

15. Polydactylous conditions in the cat and man resemble those of the *Pxpx* guinea pig, and there is some chance that polydactylous monsters of an hereditary type may be discovered in these forms.

LITERATURE CITED

- BAGG, H. J. 1929 Hereditary abnormalities of the limbs, their origin and transmission. *Am. J. Anat.*, vol. 43, pp. 167-219.
- BATESON, W. 1894 Materials for the study of variation. Macmillan, London.
- BEAN, A. M. 1929 A morphological analysis of the foot abnormalities occurring in the descendants of x-rayed mice. *Am. J. Anat.*, vol. 43, pp. 221-246.
- BROMAN, I. 1911 Normale und abnorme Entwicklung des Menschen. J. F. Bergmann, Wiesbaden.
- CASTLE, W. E. 1906 The origin of a polydactylous race of guinea pigs. *Pub. Carnegie Inst. of Washington*, no. 49, pp. 15-27.
- DAVIDSON, A. J. 1918 A case of congenital deformity of the hands, supernumerary toes, and absence of tibiae. *Am. J. Roentgenology*, vol. 5, pp. 434-436.
- HARMAN, M. T., AND O. B. SAFFRY 1934 The skeletal development of the anterior limb of the guinea pig, *Cavia cobaya* Cuv., from the 25-day embryo to the 161-day post natal guinea pig. *Am. J. Anat.*, vol. 54, pp. 315-331.
- HOVELAQUE, A. 1920 Anatomie et morphogenie d'une anomalie héréditaire des membres abdominaux. *Bull. Biol. de France et de Bel., Suppl.*, T. 3, pp. 1-156.

- PFITZNER, W. 1898 Beiträge zur Kenntniss der Missbildungen des menschlichen Extremitätenskelets. IV. Eine Familie mit erblichen Missbildungen an den Extremitäten. *Morph. Arbeit.*, Bd. 8, S. 304-322.
- PILMANIS, E. 1933 Hiperdaktilija. *Latvijas Arstu Zurnal* 9. Abh., 23s. Quoted from *Anat. Bericht.*, Bd. 29, S. 176.
- PUNNETT, R. C., AND M. S. PEASE 1929 Genetic studies in poultry. VII. Notes on polydactyly. *J. Genetics*, vol. 21, pp. 341-366.
- SCOTT, J. P. 1937 The embryology of the guinea pig. I. A table of normal development. *Am. J. Anat.*, vol. 60, pp. 397-432.
- 1937 a The embryology of the guinea pig. III. The development of the polydactylous monster. A case of growth accelerated at a particular period by a semi-dominant lethal gene. *J. Exp. Zool.*, vol. 77, pp. 123-157.
- SCOTT, J. P., R. J. WEDDING AND W. R. KIRTLEY The anatomy of a polydactylous cat. (Unpublished.)
- SNYDER, L. H. 1929 A recessive factor for polydactylism in man. *J. Hered.*, vol. 20, pp. 73-77.
- STOCKARD, C. R. 1930 The presence of a factorial basis for characters lost in evolution: the atavistic reappearance of digits in mammals. *Am. J. Anat.*, vol. 45, pp. 345-377.
- SVERDRUP, A. 1922 Postaxial polydactylism in six generations of a Norwegian family. *J. Genetics*. vol. 12, pp. 217-240.
- WRIGHT, S. 1934 Polydactylous guinea pigs. *J. Hered.*, vol. 25, pp. 359-362.
- 1934 a Genetics of abnormal growth in the guinea pig. *Cold Spring Harbor Symposia on Quantitative Biology*, vol. 2, pp. 137-147.
- 1935 A mutation of the guinea pig, tending to restore the pentadactyl foot when heterozygous, producing a monster when homozygous. *Genetics*, vol. 20, pp. 84-107.

ABBREVIATIONS

a.c., anterior commissure	pal., palatine
a.v., arbor vita	par., parietal
ba., basilar artery	ph., or phal., phalanx
b.i., inner basal pad	pi., pineal body
b.o., outer basal pad	pis., pisiform
b.occ., basioccipital	po., pons
bul., bulla	p.s.p., presphenoid
c., etc., first carpal, etc.	pte., pterygoid
c.c., corpus callosum	px., premaxilla
cen., central	rad., radius
cere., cerebellum	rade., radial
d., distal pad	rad.ep., epiphysis of radius
di, diencephalon	rad.s., radial sesamoid
fib., fibula	sph., sphenoid
fibe., fibular	spl., splint
fib.ep., epiphysis of fibula	sq., squamosal
f.M., foramen of Monro	s.s., sagittal sinus
fr., frontal	st ₄ , fourth sternebra
hem., cerebral hemisphere	sup., supra-orbital ridge
h.pa., heel pad	t ₁₁ , toe eleven
hu., humerus	tV etc., true fifth digit, etc.
in., incisor	ta ₄ , etc., fourth tarsal etc.
inp., interparietal	tib., tibia
jg., jugal	tibe., tibial
lac., lacrimal	tib.ep., epiphysis of tibia
man., mandible	tib.s., tibial sesamoid
max., maxilla	t.p., toe pad
m.c., middle commissure	tu., tubercle
McI, etc., first metacarpal, etc.	ul., ulna
med., medulla	ule., ulnar
men., mesencephalon	ulep., epiphysis of ulna
men.c., cavity of mesencephalon	vo., vomer
MtII, etc., second metatarsal, etc.	ve., vermis
na., nasal	ven., common ventricle
o.c., optic chiasma	3ven., third ventricle
occ., occipital	

PLATE 1

EXPLANATION OF FIGURES

Figures 2 to 4. Front feet of newborn ID animals from palmar surface. $\times 2$.

2 Monster.

3 Heterozygous polydactyl, with well-developed thumb.

4 Homozygous normal. The disparity in size is accidental.

Figures 5 to 7. Skeletons of hand and forearm of newborn animals, drawn from Spalteholz preparations. Dorsal aspect. $\times 2$.

5 Monster. The bones are shown spread apart. ID.

6 Heterozygous polydactyl with sixth toe at base of thumb. Note separation of metacarpal I from distal epiphysis. ID.

7 Normal. I.

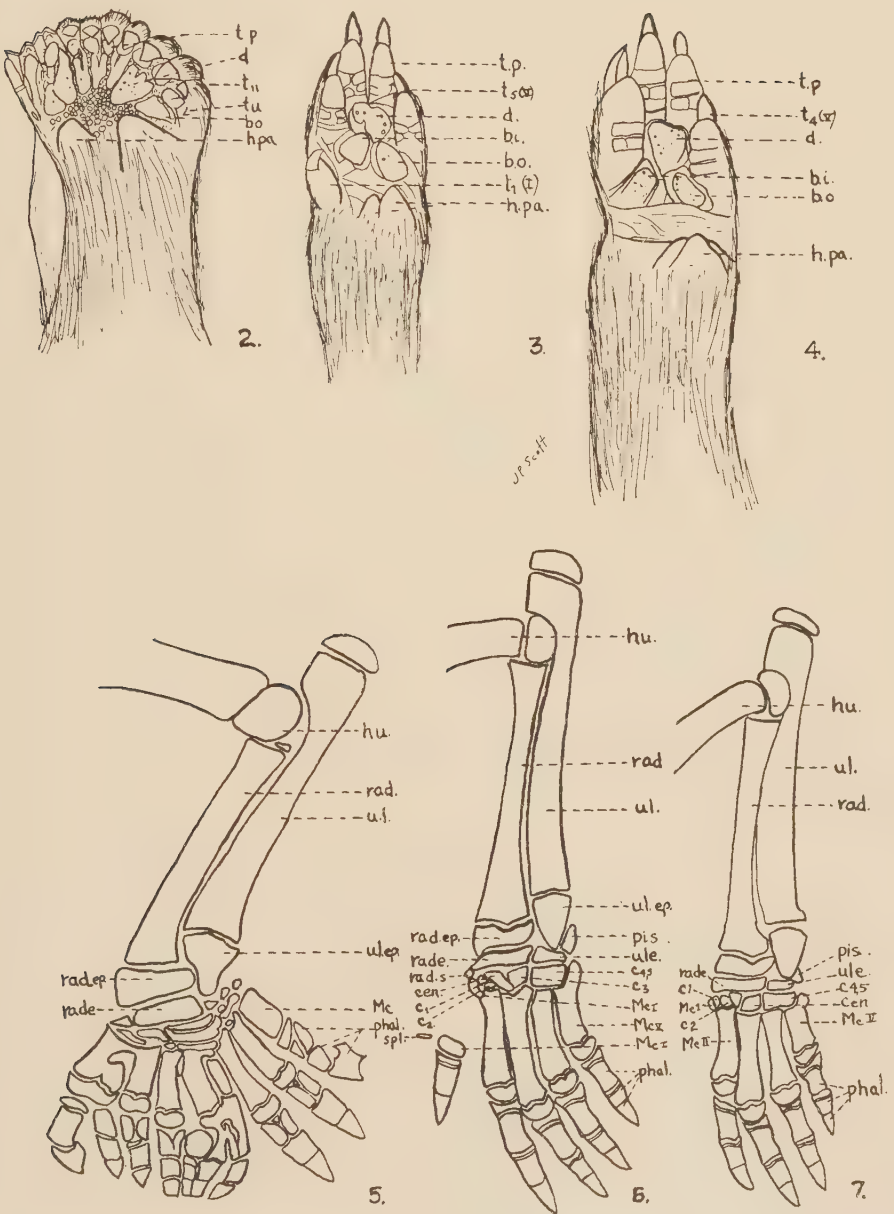


PLATE 2

EXPLANATION OF FIGURES

Figures 8 to 10. Hind feet of animals shown in figures 2 to 4, from plantar surface. $\times 2$.

8 Monster.

9 Heterozygous polydactyl with usual type of hallux and well-developed little toe.

10 Homozygous normal, showing multiple factor type of polydactyly.

Figures 11 to 13. Skeletons of hind feet from same preparations as in figures 5 to 7. Dorsal aspect. $\times 2$.

11 Monster. The bones are spread apart to avoid superposition.

12 Heterozygous polydactyl with hallux.

13 Normal.



PLATE 3

EXPLANATION OF FIGURES

Figures 14 to 16. Skull of newborn monster with harelip and cleft palate but brain not protruding. Severe microphthalmia on left side only. $\times 1\frac{1}{2}$.

- 14 Left side.
- 15 Right side.
- 16 Ventral.

Figures 17 to 19. Ossified portions of sterna of preparations shown in figures 2 to 4. $\times 1$.

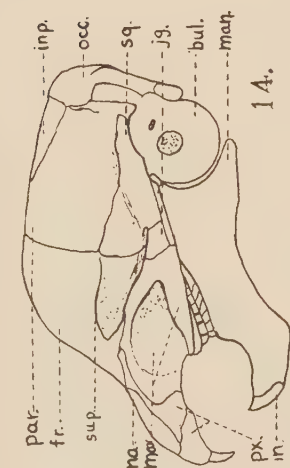
- 17 Monster.
- 18 Heterozygote.
- 19 Normal.

Figures 20 and 22. Brain of newborn monster. ID. $\times 1\frac{1}{2}$.

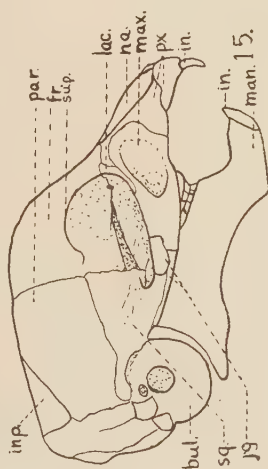
- 20 Dorsal.
- 22 Sagittal section.

Figures 21 and 23. Brain of non-polydactylous litter mate of the preceding. $\times 1\frac{1}{2}$.

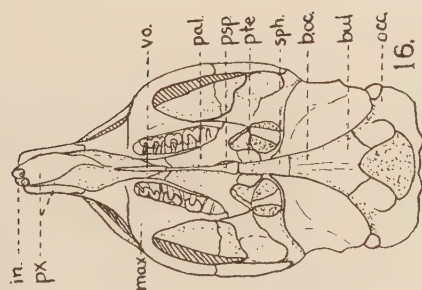
- 21 Dorsal.
- 23 Sagittal section.



14.

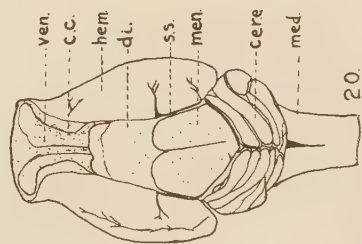


15.



16.

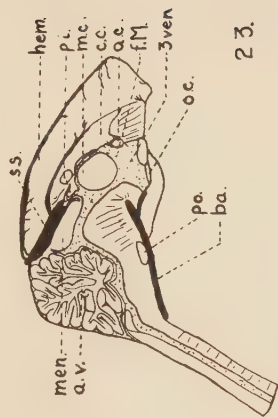
16.



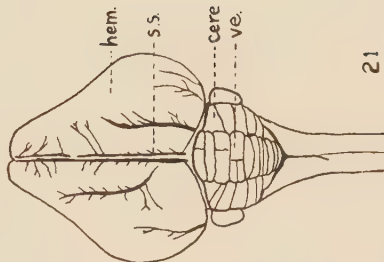
20.



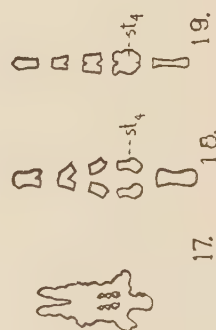
22.



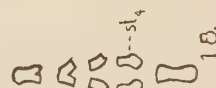
23.



21.



17.



18.



19.

THE EMBRYOLOGY OF THE GUINEA PIG

III. THE DEVELOPMENT OF THE POLYDACTYLOUS MONSTER. A CASE OF GROWTH ACCELERATED AT A PARTICULAR PERIOD BY A SEMI-DOMINANT LETHAL GENE ¹

J. P. SCOTT

Whitman Laboratory, The University of Chicago

THIRTY-THREE FIGURES

INTRODUCTION

It has long been realized that the limitations of both physiological genetics and experimental embryology can be defined by the gap in knowledge between the evidence for the existence of hereditary factors and the observation of their final visible effects. That is, the chief concern of these two sciences is the problem of how hereditary factors act, singly in genetics and collectively in embryology. One science attempts to vary the causal agents by selection, the other to modify their effects by environmental interference. An anomaly produced by a single gene provides a controlled variation of heredity comparable to the controlled variations of environment used in experimental embryology and has been so employed in many recent studies.

Such an anomaly was discovered and described by Wright ('34, '34 a, '35), who reported that in the guinea pig the semi-dominant lethal factor *Px* produced extra toes in the heterozygous condition and a polydactylous monster in the homozygous condition. The anatomy of those monsters which lived up to the time of birth has since been described in detail (Scott, '37 a). All organ systems except the circulatory, reproductive and endocrine (the last not studied) showed serious

¹ This paper comprises the third part of a doctoral thesis in zoölogy submitted to the faculty of The University of Chicago. The author is indebted to Dr. Sewall Wright for suggesting the problem and for advice and criticism.

abnormalities. These were attributed to an arrest of morphogenesis plus an alteration of relative growth rates.

Wright ('35) has suggested that the primary effect of the *PxPx* genes is a general inhibition of development at a particular moment, following Stockard's theory. Such a general inhibition could be produced by interference with general processes going on in all cells (metabolism), or with specific processes going on in certain cells having control over general processes.

However, unlike most monsters produced by inhibitory influences, this type exhibits excessive growth as well as suppression of certain organs.

MATERIALS AND METHODS

Monstrous embryos were obtained by mating heterozygote with heterozygote within the I and ID stocks of Wright's Chicago guinea pig colony. The ID stock (Wright, '35) was produced by a cross between the D and I stocks—showing respectively multiple factor and single factor (*Pxpx*) polydactyly—and exhibits additive effects of both sets of factors. The I stock probably carries a considerable number of unfavorable hereditary qualities (table 1) and possibly other lethal factors, but there is no evidence of interference with the expression of the *PxPx* genes.

Most of the embryos were prepared by the method previously described (Scott, '37)—removal by caesarian section, fixation in Bonin's fluid, and staining in Mallory's phospho-tungstic haematoxylin. The exact copulation age was obtained for all litters and the position in the uterus of each embryo noted.

Thirty-three litters were so obtained, ranging from 11½ to 43 days copulation age. The first recognizable monster (fig. 4) was obtained at 18 days, 12 hours and 15 minutes (18-12-15). The neural folds in the region of the cervical flexure were thrown into irregular wavy lines (fig. 2). Older monsters also show microphthalmia and paddle-shaped limbs. Table 1 gives a classification of all embryos of 18½ days and over, including cross-bred non-polydactylous material. Twenty-three monsters were obtained, confirming Wright's theory

of a lethal gene (expectation = 16.5 ± 3.5). Ten litters contained living monsters, and a study of nine of these provided the basis for the present paper. The most advanced normal embryo in each of six of the nine has been described in the author's table of normal development (Scott, '37).

TABLE 1
Classification of embryos of 18½ days and over

TYPE	STOCK		
	I	ID	Random cross-bred
<i>PxPx</i>			
Dead <i>PxPx</i> monsters	6	3	0
Living <i>PxPx</i> monsters	3	9	0
Allantois a solid bud	0	1	0
Retarded 2 days	1	0	0
Total <i>PxPx</i>	10	13	0
<i>Pxpx</i> or <i>pxpx</i>			
<i>Pxpx</i> polydaetyls	4	6	0
Retarded 2 days	1	0	0
— <i>px</i> , no toes developed	6	18	24
In double decidua	1	1	0
Allantois a solid bud	0	1	0
Retarded 2 days	2	0	0
<i>pxpx</i> non-polydaetyls	2	1	17
Total not <i>PxPx</i>	16	27	41
Unclassified as to <i>Px</i>			
Possible <i>PxPx</i>	0	1	1
Retarded 2 days (doubtful)	2	0	0
Grossly abnormal, allantois not attached to placenta	2	0	0
Undifferentiated cell mass	1	0	0
Empty decidua	2	2	2
Unclassified dead	1	0	2
Total not classified	8	3	5
Total	34	43	46

The most advanced monster of each litter was compared externally and in section with its most advanced normal litter mate. The method would be unfair, there being an expectation of three times as many normal animals as monsters, were it not that litters containing no monsters were omitted, and the numbers were nearly equal (fourteen and twelve).

This method of paired comparisons controls variable intra-uterine environmental factors by selection of the most advanced embryos and inter-uterine factors by avoidance of comparisons between members of different litters. Extraneous hereditary factors were slightly controlled by inbreeding. An ideal experiment of this type should use: 1) inbred stocks, 2) exact timing, 3) identical treatment of embryos, and 4) comparison of the most advanced representative of each type within a litter. Other controls, such as age of mother and type of food, are probably not so important.

No correlation between uterine position and size or abnormality was discovered.

A TABLE OF DIFFERENCES

Living monsters may be obtained from about the middle of the period of embryonic organ formation almost to the end of the fetal period, according to the previous definition of these periods (Scott, '37). Below are given only the differences between such monsters and their most advanced normal litter mates. Differences no longer apparent at any stage are omitted without mention. The arrangement of the material follows that of the author's 'Normentafel' (Scott, '37), in which the normal animals of all but litters 20-10-20, 22-17-35, and 24-13-1 are described, these being delayed in development 1 day or longer.

TABLE 2

Differences between most advanced monstrous and normal litter mates

Age 18-12-15. Upper right (ID) (fig. 4)

a. External.

5. Sense organs. Eye less prominent; no lens pit. Nasal pit very shallow.
6. Visceral arches. Maxillary process not continuous with lateral wall of nasal pit.
7. Limb buds. Fore bud somewhat larger than normal; hind bud not defined.
8. Circulatory. Allantoic vein not visible.

b. Internal.

2. Somites. Notochord still touches roof of pharynx. Right opening between pleural and pericardial cavity larger.

3. Nervous. Telencephalon with no anterior lobes, rounded rather than heart-shaped. Diencephalon with anterior portion rounded rather than lobed. Myelencephalon with irregularly folded walls behind the fifth neuromere; many mitoses at points of folding.

Head with less prominent lobes; nasal pit shallower. Optic cup without ventral walls; lens plate rather than cup. Otic vesicle irregular in shape; ganglion VII-VIII somewhat lateral rather than medial to it.

Ophthalmic nerve not traceable to eye; mandibular branch contains cell bodies. Ganglion V lateral rather than ventral to metencephalon. Ventral portion of VII posterior rather than medial to hyomandibular pouch; hyoid branch extends farther. IX somewhat longer. Nerve XI reaches only first root. Nineteen instead of twenty-one discernible dorsal root ganglia, none as well formed as normal. Cord folded to second dorsal root.

4. Digestive. Fourth visceral pouch touches ectoderm. No lateral grooves in roof of mouth. No pouch above corner of mouth.

Trachea extremely short, bifurcating where cut off; bronchi somewhat shorter than normal; allantoic diverticulum shorter than normal.

5. Circulatory. Allantoic veins much smaller than normal; left yolk sac vein also small. Ducts of Cuvier relatively small. Branch of anterior to base of arches smaller than normal. A number of irregular sinuses below the liver.
7. Endocrine. Rathke's pouch nearly cut off, instead of being open. Aortic bodies slightly less extensive.
8. Limb buds. Fore limb bud more prominent, more rounded, about twice as thick dorso-ventrally. All adjacent somites touch the bud instead of 11-12 only. Hind limb bud less prominent.

Age 19-2-45. Upper left (I) (fig. 6)

a. External.

5. Sense organs. Nasal pit still small and shallow. Eye small; barely visible externally.
6. Visceral arches. Maxillary process still does not reach nasal pit.
7. Limb buds. Fore bud not elongate.

b. Internal.

2. Somites. Notochordal sheath fibrous, not vacuolated; anterior tip gone.
3. Nervous. Telencephalon with no anterior lobes, not heart-shaped, no posterior lobes. Diencephalon with anterior tip inclined laterally and irregularly folded. Metencephalon with deeper and more pronounced folds. Myelencephalon with walls strongly folded as far forward as the fourth neuromere.

Nasal pit shallow, small and lateral; no lateral wall; not reached by maxillary process. No trace of nerve I. Optic cup flattened; ventral wall does not touch ectoderm. Endolymphatic duct open; otic vesicle of irregular shape and partly medial to ganglia VII, VIII.

Ganglion V touches ectoderm; ganglion VII separate from VIII. Nerve XII fuses with first spinal. Only seventeen instead of twenty-four dorsal root ganglia discernible. Cord folded to third spinal nerve; folds much more pronounced than previous stage.

4. Digestive. Visceral arches larger; ectodermal thickening of maxillary process is lateral instead of ventral; this process extends to eye and partly covers it. Roof of mouth folded and mouth cavity small. Hyomandibular pocket unusually long.

Trachea and bronchi both short, being contained in distance of 1 somite; stomach begins about 1 somite anterior to that of normal. Liver occupies most of coelom, is more anterior but about normal size. Proliferation from end of bile duct present. Allantoic portion of gut small and wide.

5. Circulatory. Irregular branching sinuses from posterior pocket of sinus venosus below the liver.
6. Excretory. Urinary ridge somewhat flattened on tip.
7. Endocrine. Rathke's pouch cut off from ectoderm.
8. Limb buds. Fore bud thicker and more rounded than normal; hind bud the same, less distinctly formed.

Age 19-16-55. Upper left (ID) (fig. 8)

a. External.

2. Form. Body flexed about 135 instead of 120°, tail not pointed; brain still flexed in region of metencephalon.
3. Somites. Thirty-six instead of thirty-eight visible.
4. Nervous. Cerebral lobes less prominent.
5. Sense organs. Nasal pit shallow, eye minute.
6. Visceral arches. Maxillary process still does not reach normal length; hyoid bilobed.
7. Limb buds. Neither limb bud is elongate.

b. Internal.

2. Somites, thirty-seven. Ventral mesentery of fore gut still nearly entire. Pleural and pericardial cavities widely instead of narrowly joined.
3. Nervous. Intermediate layer less extensive; no oval tract in cord. Telencephalon without anterior lobes, or central ridge; small posterior lobe; walls thickest laterally rather than ventrally; very slight indication of corpus striatum; never heart shaped in section. Diencephalon with anterior lobe expanded, folded irregularly, not tongue shaped, no thin-walled portion. Ventral cavity widely expanded at eyes. Neuromeres more prominent. Walls of myelencephalon more strongly folded than in previous stage.

Nasal pit shallow, without lateral wall, far back from end of head, not reached by maxillary process. Lens cup instead of vesicle; ventral side of optic cup does not reach ectoderm; optic cup compressed dorso-ventrally; maxillary arch somewhat lateral to it. Otic vesicle with no cochlear pit but now rounded and nearly posterior to ganglia.

No indication of nerve I. Nerve III does not reach eye. Ganglion V lateral to metencephalon and touching ectoderm; branches smaller than normal. Nerve VIII more lateral to otic vesicle. Ganglion IX with expanded, non-fibrous root. Connection between jugular ganglion and ganglion nodosum small. Twenty-four instead of twenty-five dorsal root ganglia. Dorsal portions of walls of cord folded to third dorsal ganglion, with many mitoses.

4. Digestive. Maxillary process rotated so that it faces laterally. Mandibular process strongly grooved throughout; roof of mouth with shallow only lateral grooves. Upper edge of mandibular arch strongly undercut.

Trachea and bronchi extend over about 1 somite, both being shorter than the normal. Laryngeal groove not continuous with the tracheal groove. Mesentery of the hind gut more sharply bent, there being no room for it in the restricted coelom; the small portion of the hind gut very short.

5. Circulatory. Allantoic artery has medial root. Left allantoic vein smaller than yolk sac vein instead of vice versa. Connection of left duct of Cuvier to heart very small; ducts unusually large.
6. Excretory. Urinary ridge still hangs down separately from mesentery of gut.
7. Endocrine. Rathke's pouch with slight connection to the exterior instead of entirely cut off.
8. Limb buds. Fore bud thicker, more rounded, projects more laterally, with no definite dorsal edge. Somite 12 still present adjacent the bud. Hind bud a flat, round swelling; no nerves developed; no ectodermal thickening at tip.

Age 20-10-20. Lower left (I) (fig. 10)

(Compared with a litter mate which had a solid, unattached allantois and which was developed only slightly beyond the preceding normal stage.)

a. External.

3. Somites. Thirty-five instead of thirty-eight visible.
4. Nervous. Visible bulge in region of cervical flexure.
5. Sense organs. Eye small.
6. Visceral arches. Maxillary process enlarged at base.
7. Limb buds. Both larger and wider than normal; no indication of hand.

b. Internal.

2. Somites, thirty-eight. Almost no umbilical pouch developed.
3. Nervous. Telencephalon still undivided, no posterior lobes; only slight thickening of ventral wall. Diencephalon with anterior lobe pressed against outer ectoderm, with irregular folds and processes; shows irregular tongue-shape. Metencephalon with two pronounced neuromeres. Myelencephalon with dorsal and posterior walls thrown into strong irregular folds; neuromeres accentuated, especially second and fourth. Fiber tracts in neuromere 2.

Nasal pits shallower and shorter than normal. Optic cup small, far from ectoderm; small lens vesicle connected to outer ectoderm by string of cells. Otic vesicle irregular in shape.

Nerve III does not reach eye. Ganglia VII-VIII still fused but differentiated. Nerve XII passes to posterior wall of anterior cardinal. Nerve X reaches ventral wall of pharynx. Thirty-three instead of thirty dorsal root ganglia discernible.

4. Digestive. Maxillary process rotated to face laterally at level of eye. Mandibular deeply underreut dorsally. Indication of fifth visceral arch present.
Tracheal groove not interrupted. Lobes of liver dorsally flattened; proliferation from bile duct. Hind gut with short mesentery.
5. Circulatory. All aortic arches small in size; left dorsal aorta much larger than right posteriorly. No medial branch to allantoic arteries. Left allantoic vein much larger than yolk sac vein; does not break up in liver. Anterior cardinal sinus very large; most vessels relatively smaller than normal.
6. Excretory. Urinary ridge projects ventrally instead of laterally. Only one open tubule instead of two.
7. Endocrine. Both oral and neural portions of hypophysis show rapid growth; oral portion cut off, has large lumen.
8. Limb buds. Enormously thickened at base.
9. Skeletal. Centra visible to about somite 24 instead of 20.
10. Genital. Genital thickening next to mesentery about somite 15, not present on normal.
11. Miscellaneous. Two pimples on side of head formed by knot of tissue beneath ectoderm.

Age 21-15-15. Upper right (ID) (fig. 12)

a. External.

2. Form. Still with extreme cervical flexure.
3. Somites. Thirty-six visible and distinct instead of disappearing.
4. Nervous. Divisions of brain still externally distinct; telencephalon small.
5. Sense organs. Eye minute; nasal pit longer than normal; no external auditory meatus.
6. Visceral arches. Narrow instead of wide premaxillary process. Mandibular and maxillary processes still form a rough square. Hyoid distinct, and cervical sinus open wider than normal.
7. Limb buds. Wider and shorter than normal; no hand; hind bud not elongate.

b. Internal.

2. Somites. All distinguishable instead of last few only; dorsal edge of dermatome clear at somite 6 instead of 30. Fibrous sheath of notochord extends to somite 21 instead of 32. Umbilical pouch small, not reached by gut. Opening between pleural and pericardial coeloms more extensive.
3. Nervous. Telencephalon thin walled, heart-shaped, no hemispheres, only small posterior lobes. Diencephalon with wide anterior lobe irregularly folded and vascularized around tip; widely expanded at eyes. Walls of brain and cord nowhere extensively thickened. Myelencephalon with posterior portion widely spread; neuromeres prominent; irregularly folded. Metencephalon and myelencephalon not widely expanded at junction.

Nasal pit with moderately long closed portion which reaches to end of maxillary process, which is entirely lateral; no sign of nasal septum; ectoderm of mouth and nasal pit continuous throughout. Eye simply a small blind pocket similar to original outgrowth; no lens, no sign of growth, no fibers. Otic vesicle irregularly rounded instead of flattened.

Nerve I small. Anlagen of eye muscles barely visible. Ganglion V oval instead of round and lateral to metencephalon. Maxillary and ophthalmic branches shorter than normal. Nerve XI irregular, does not extend so far. Nerve XII passes to junction with X only. Vagus passes as far as origin of trachea and not to stomach.

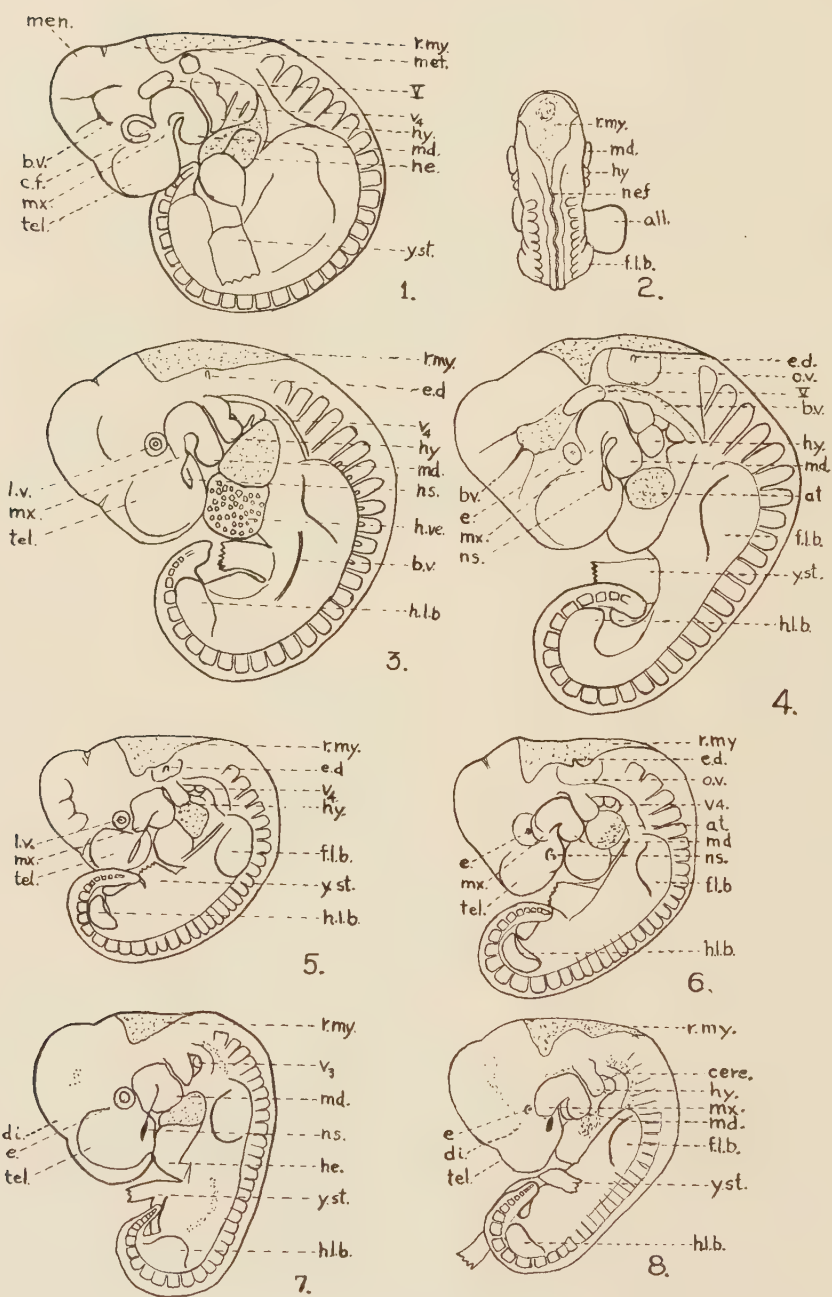
4. Digestive. Maxillary processes not rotated. Ends of mandibular processes still knobbed; mandibular arch undercut dorsally. Hyoid distinct from mandible. No skeleton in jaw, no tongue elevation. A projection from the roof of the mouth anterior to the hypophysis. Mouth cavity flat and wide.

Trachea not cut off immediately behind the larynx; laryngeal and tracheal grooves continuous. Bronchi with one branch instead of two. Stomach small, nearly upright instead of lying on side. No coils of gut in umbilical pouch. Mesentery of hind gut bent to left. Perineal tubercle small; part of gut still in tail.

ABBREVIATIONS FOR ALL FIGURES ²

all., allantois	met., metencephalon
a.mt., auditory meatus	m.h., milk hill
at., atrium	mx., maxillary process
bl., bleb (watery)	nar., nostril
b.v., blood vessel	ne.f., neural folds
cere., cervical sinus	nip., nipple
c.f., choroid fissure	n.s., nasal pit
di., diencephalon	o.v., otic vesicle
e., eye	pap., papillae (of monsters)
e.d., endolymphatic duct	p ₂ , etc., projections (anlagen of digits)
e.l., eyelid	pe.t., perineal tubercle
ex., excrescence	pin., pinna
f.l.b., fore limb bud	p.p., premaxillary process
f.M., foramen of Monro	r.my., roof of myelencephalon
g., gut	s.h., shoulder hump
he., heart	som., somite
he.v., ventricle of heart	spi., spine
h.l.b., hind limb bud	tel., telencephalon
h.r., haemal ring (eye)	th., thickening (folded neural tube)
hy., hyoid arch	umb., umbilical pouch
i.v., intervertebral space	v ₄ , etc., fourth visceral arch, etc.
j., jaw	ves., vesicular tissue of umbilical pouch
l.v., lens vesicle	vib., vibrissa
m., mouth	y.st., yolk stalk
md., mandibular process	V, Trigeminal ganglion
men., mesencephalon	

² Figures 1, 3, 5, 7, 11, 17 and 21 are reproduced from part I of this series for purposes of comparison.



5. Circulatory. Aortic septum not complete, not rotated as much as normal. Walls of arteries not so far differentiated. Still some nucleated blood cells. Caudal aortae paired.
6. Excretory. Mesonephric duct still connects with metanephric enlargement, which is smaller than normal.
7. Endocrine. Oral portion of hypophysis not divided; third portion present as hollow tube; both this and oral portion found in projection from roof of mouth. Aortic bodies not as prominent; no signs of differentiation.
8. Limb buds. Anterior bud projects more laterally; palmar portion short, pointed; bud short and stubby compared to normal, thicker at base. Somite 12 bud still visible but tissue transformed. Hind bud has no ectodermal ridge, only slightly undercut.
9. Skeletal. Centra distinguishable to ganglion 27 instead of 33. Anlagen of cartilage bones much less developed, practically no indication of skull.
10. Genital. Genital ridge with only a cortical thickening; much smaller than normal. No mammae present.

Age 22-17-35. Right (ID) (fig. 14)

a. External.

1. Extra-embryonic. Umbilical pouch narrow.
2. Form. Cervical flexure still nearly complete.
3. Somites. Anterior somites still distinguishable.
4. Nervous. Cerebral lobes smaller; diencephalon projects dorsally.
5. Sense organs. Eye small; nasal pit larger.
6. Visceral arches. Still retain identity, though in process of transformation.
7. Limb buds. Both hand and hind bud wider and shorter than normal.

b. Internal.

2. Somites. Mouth of umbilicus widely open; pleural folds short.
3. Nervous. Telencephalon with only extreme tip divided; walls relatively thin, corpora striata compressed; foramen of Monro about twice as wide as normal; no choroid plexus developed from posterior lobes. Diencephalon enormously enlarged, especially dorsally, and extending nearly to tip of telencephalon; folded anteriorly. Mesencephalon with relatively thicker wall. Metencephalon larger than normal. Myelencephalon relatively longer, walls folded irregularly except in extreme dorsal portion; prominent neuromeres. Cord folded to sixth ganglion.

Fig. 1 Normal embryo (lower right). Age 17-12-40. ID stock. $\times 10$.

Fig. 2 Monster with allantois a solid bud. Dorsal view. Age 19-16-55. ID stock. $\times 5$. Note distortion of the neural folds.

Fig. 3 Normal embryo (lower right). Age 18-12-15. ID stock. $\times 10$.

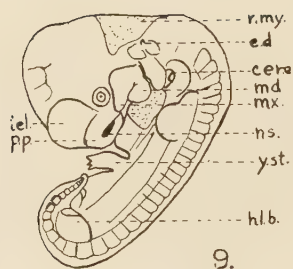
Fig. 4 Monstrous embryo (upper right). Litter mate of 3. $\times 10$.

Fig. 5 Normal embryo (second left). Age 19-2-45. I stock. $\times 5$.

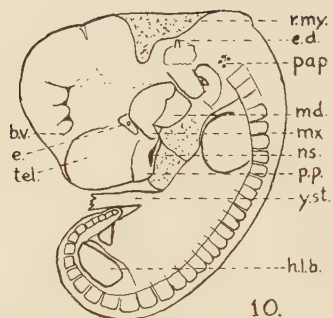
Fig. 6 Monstrous embryo (upper right). Litter mate of 5. $\times 5$.

Fig. 7 Normal embryo (middle left). Age 19-16-55. ID stock. $\times 5$.

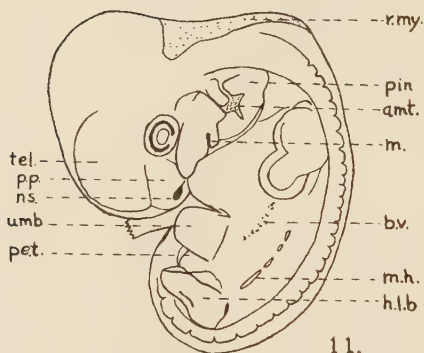
Fig. 8 Monstrous embryo (upper right). Litter mate of 7. $\times 5$.



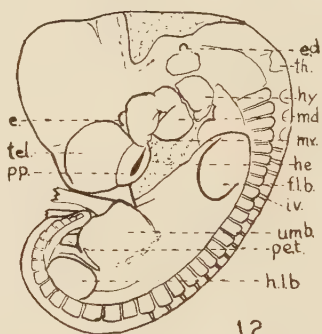
9.



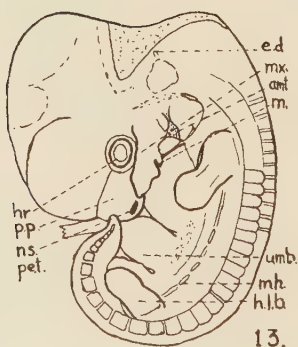
10.



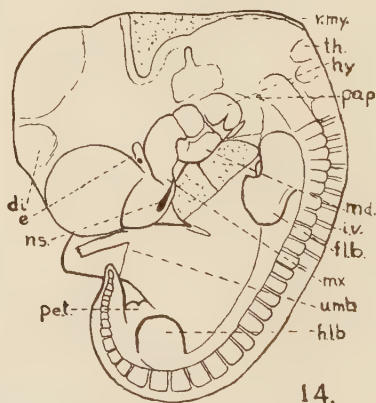
11.



12.



13.



14.

Nasal pits slightly deformed, smaller, more widely open. Eyes are compressed, thin walled tubes, directed slightly dorsally and not touching outer ectoderm; left smaller and nearly cut off from brain. Endolymphatic duct touches ectoderm.

Ganglion V oval in section instead of round. Nerve X passes only as far as bronchi. Thirty-two instead of thirty-one dorsal root ganglia.

4. Digestive. Maxillary process not rotated, forms prominent lateral projection with mandibular process; ectodermal thickening ventral. Mandibular with club-shaped ends. Hyoid slightly larger than normal; cervical sinus larger than normal with two instead of one arch inside it. Vesicle not cut off fourth pouch. Laryngeal groove wide open, not round posteriorly.
- Stomach smaller, not tipped over so far. Cloaca more widely open.
5. Circulatory. Larger interatrial foramen. Systemic and pulmonary aortas less completely separate; pulmonary artery present. Anterior cardinals relatively larger than normal.
6. Excretory. First tubules about ganglion 8 instead of 15.
7. Endocrine. Posterior pockets of oral portion of hypophysis very shallow; still slight connection to mouth; third portion on right instead of medially. Aortic bodies relatively smaller.
8. Limb buds. On right fore bud a dorsal papilla; top and bottom of hand rounded instead of straight. Both buds thicker than normal.
10. Genital. Genital region relatively smaller; thickening less extensive.
11. Miscellaneous. Small papilla on right side of head.

Age 23-16-5. Middle right (ID) (fig. 18)

(The abnormalities are unusual in several respects.)

a. External.

1. Extra-embryonic. Umbilical pouch narrow, no vesicular structure surrounding it.
2. Form. Cervical flexure about 30° greater than normal.
4. Nervous. The enlarged diencephalon forms a projection on top of the head.
5. Sense organs. Nasal groove still remains. Eye reduced. External ear less prominent.
6. Visceral arches. Maxillary process still present; lips not present; vibrissal papillae enlarged.
7. Limb buds. Front foot not rotated; toes less differentiated. Both feet shorter than normal and much wider.

b. Internal.

Fig. 9 Non-*PxPx* embryo with allantois a solid bud. (upper right). Age 20-10-20. I stock. $\times 5$.

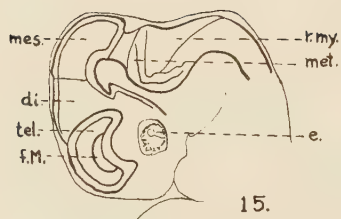
Fig. 10 Monstrous embryo (lower left). Litter mate of 9. $\times 5$.

Fig. 11 Normal embryo (lower right). Age 21-15-15. ID stock. $\times 5$.

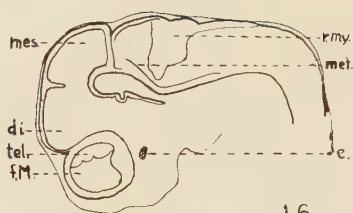
Fig. 12 Monstrous embryo (upper right). Litter mate of 11. $\times 5$.

Fig. 13 Normal embryo (upper left). Age 22-17-35. ID stock. $\times 5$.

Fig. 14 Monstrous embryo (right). Litter mate of 13. $\times 5$.



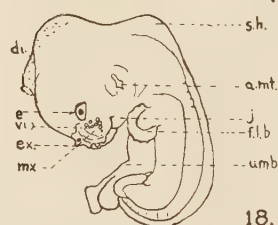
15.



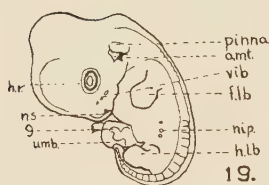
16.



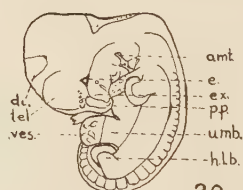
17.



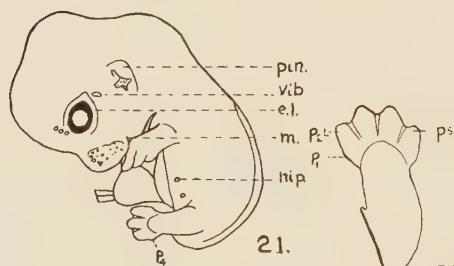
18.



19.



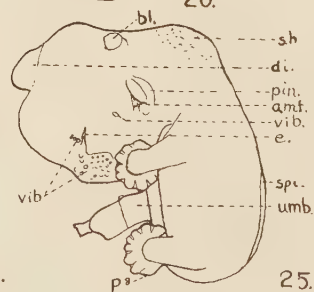
20.



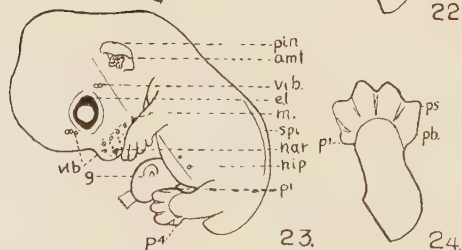
21.



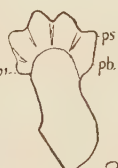
22.



25.



23.



24.



26.

2. Somites. Coelom and umbilical pouch less extensive than normal, organs tightly packed. Cavity of omental bursa smaller. Lung buds project farther back than pleural folds; peritoneal recesses persist lateral to them.
3. Nervous. Telencephalon with walls much folded; the two halves forced far apart except anteriorly; no olfactory lobes; no dense layer in cortex; corpora striata folded. Diencephalon greatly enlarged; anterior half invaginated, pushing the hemispheres apart; posterior portion pushed through to outside; third ventricle wider than normal, the walls not touching; whole slightly asymmetrical. Tract from corpora striata less clearly demarcated; posterior tract does not extend to mesencephalon. Mesencephalon with folded walls, the dorsal portion folded over to the left. Metencephalon dorso-ventrally compressed, walls curved irregularly. Myelencephalon enlarged and folded dorsally; retains irregular folds in region of neuromeres. Cord folded down to ganglion 13 and from ganglion 23 to 27; walls less extensively fused than normal.

Nasal passages smaller and more rounded; Jacobson's organ small; epithelium lifted by lymph space in one place. Left eye nearly normal in size; right smaller; left directed nearly ventrally; both located more medially than normal; lens fibers not straight; whole orbital space, including eye muscles, reduced. Semicircular canals present only as small blind outgrowths from utriculus, which is retarded.

Nerve I nucleated, unlike normal. IX and X separate, unlike normal. Anterior ganglia more distinct than in normal.

4. Digestive. Little epidermal tissue in vibrissal papillae; tip of nose covered with knobs. Groove in front of mouth about twice as wide as normal. Tip of lower jaw heavier; mouth cut back farther; tip of tongue not free; mouth cavity smaller. Remnant of visceral arches dorsally; second pouch still present; remnant of cervical sinus.

Stomach and liver crowded; the liver sinuses distinctly smaller than the normal. One ventral bend of gut in umbilicus instead of two. Meckel's diverticulum directed ventrally instead of anteriorly. Only one right lobe of liver. Hind gut squeezed against ventral body wall; rectal enlargement smaller than normal.

Fig. 15 Brain of figure 13, seen by transparency. $\times 5$.

Fig. 16 Brain of figure 14, seen by transparency. $\times 5$.

Fig. 17 Normal embryo (lower right). Age 23-16-5. ID stock. $\times 2\frac{1}{2}$.

Fig. 18 Monstrous embryo (middle right). Litter mate of 17. Slightly injured during removal from uterus. $\times 2\frac{1}{2}$.

Fig. 19 Normal embryo (upper left). Age 24-13-1. I stock. Development retarded about 2 days. $\times 2\frac{1}{2}$.

Fig. 20 Monstrous embryo (lower left). Litter mate of 19. $\times 2\frac{1}{2}$.

Fig. 21 Normal embryo, *p p p* (lower right). Age 26-3-45. ID stock. $\times 2$.

Fig. 22 Right front foot of figure 21. $\times 5$.

Fig. 23 Normal embryo, *P x p* (left). Litter mate of 21. $\times 2$.

Fig. 24 Right front foot of figure 23. $\times 5$.

Fig. 25 Monstrous embryo, *P x P x* (upper right). In dying condition. Litter mate of 21. $\times 2$.

Fig. 26 Right front foot from figure 25. $\times 5$.

5. Circulatory. Left common cardinal makes considerable posterior bend to join right atrium. Yolk sac artery small, gives off branches to omentum, unlike normal; does not come into contact with vein as in normal. Right allantoic artery larger than left.

Anterior cardinal veins show connection with lymph spaces of neck, which have blood in them. Post cardinals disappear about ganglion 14 instead of 16. Post cava, allantoic and yolk sac veins small, possibly caused by loss of blood. Post cava not connected with post cardinals. Blood found extravasated lateral to mesencephalon.

6. Excretory. Wolffian duct larger than in normal; lumen not obliterated.
7. Endocrine. Cavity of infundibulum larger than normal and empty.
8. Limb buds. Ossification behind normal. Scapula smaller, of abnormal shape; ulna not laid down; evidence of supernumary digits in hand. Femur not ossified. Shape of buds different.
9. Skeletal. Neural arches wider apart; vertebrae ossified to ganglion 24 instead of 28; ribs not so well ossified and do not pass as far ventrally as normal.
10. Genital. Mullerian duct reduced; gonad somewhat larger. No nipples.
11. Muscles. Possibly not so well differentiated in limb buds.

Age 24-13-1. Lower left (I) (fig. 20)

a. External.

2. Form. Cervical flexure about 30° greater than normal.
3. Somites. Slightly more prominent.
4. Nervous. Region of cerebrum much reduced.
5. Sense organs. Nasal pit not clearly outlined; eye minute.
6. Visceral arches. Maxillary and premaxillary processes not fused; hyoid not transformed.
7. Limb buds. Feet shorter and wider than normal.

b. Internal.

2. Somites. No neck to umbilical pouch; no connection to extra-embryonic coelom. Tip of lung projects beyond pleural fold; lengthy lateral peritoneal recess. Pleural and pericardial coeloms still continuous.
3. Nervous. Telencephalon irregularly folded throughout; posterior lobes small; no differentiation of cortex. Diencephalon enormously enlarged dorsally, not invaginated, folded anteriorly. No pocket beneath corpora striata. Mesencephalon with relatively thin wall, cavity rounded rather than oval. Metencephalon irregularly folded, cavity wide. Myelencephalon strongly folded dorsally; neuromeres accentuated.

Nasal passage round; that on left shorter than right; tear gland where eye should be. Left eye a flattened vesicle, not connected with brain, not differentiated; right slightly larger, connected with brain, differentiated; no fibers to brain from retina. Ear canals not developed; an unattached vesicle between brain and ear on right; otic vesicle smaller than normal.

Lower ganglion of IX instead of upper touching X. Vagus does not extend as far as posterior end of stomach. Phrenic nerve small, more lateral.

4. Digestive. Maxillary process not rotated; no preoral groove. Tooth grooves very small; tip of lower jaw heavy; tongue not undercut; salivary glands small. Cervical sinus still connected to outside, with remains of two visceral grooves. Visceral pouches slightly larger.
Laryngeal groove open; branches of bronchi slightly less extensive. Gall bladder small; muscular layer of duodenum does not extend to umbilicus; liver with only one lobe on right; rectal portion of gut somewhat smaller than normal.
5. Circulatory. Heart not enclosed in body wall. Left common cardinal makes a posterior loop instead of entering heart directly. No mesenteric, internal iliac or femoral arteries. Anterior cardinals unusually large; no lateral lymph spaces. Post cava not connected with left post cardinal.
6. Excretory. Tubules appear before end of lung bud. Metenephros unbranched.
7. Endocrine. Oral portion of pituitary below tip of infundibulum.
8. Limb buds. Bones of fore bud unossified, appearing as thickenings; none in hind bud.
9. Skeletal. Centra ossified to ganglion 16 instead of 21. Heads of ribs, scapula, humerus, radius, ulna, bones of nasal septum all unossified, unlike normal.
10. Genital. No mullerian duct; genital thickening longer than normal. No nipples.
11. Muscles. Not distinguishable in shoulder, upper arm and near vertebrae.

Age 26-3-45. Upper right (ID) (fig. 25)

a. External.

1. Extra-embryonic. Umbilical pouch narrow; no vesicular tissue.
2. Form. Cervical flexure about 30° greater than normal; hump in this region accentuated.
4. Nervous. Diencephalon projects through top of head.
5. Sense organs. Eye small; lids reduced.
6. Visceral arches. Pinna less developed; face not as smooth as normal; distribution of vibrissae abnormal.
7. Limb buds. Front foot not rotated 90°; extra digits present; feet paddle-shaped.

b. Internal.

2. Somites. Wolffian duct with thick mesentery; only about half of bladder in coelom. Umbilical pouch shorter than normal, with no anterior pocket. Pericardium still joined to body wall about halfway up sides. Post cava much smaller than normal.
3. Nervous. Cerebrum heavily folded, lumen practically eliminated; olfactory lobe small; dense layer of cortex thin. Diencephalon and choroid plexus spread out flat between hemispheres; slightly folded to right posteriorly, the roof being folded throughout. Enlarged mesencephalon is folded back over the metencephalon, which is squeezed flat between it and myelencephalon; roof of mesencephalon folded. Myelencephalon folded in region of neuromeres (now gone) and on posterior dorsal edge;

extensive lesion under the skin lateral to it. Germinal layer thickened beneath folds. Cord narrow; dorsal and ventral horns not developed; less differentiation between germinal and intermediate layers dorsally; folded as far as ganglion 5 and from ganglion 25 posteriorly.

Nasal passage small in diameter; internal nares large. Eye a deep, narrow pit, papilla in center; behind this small lens and brown pigmented vesicle; no connection to brain; no lens on left; eye muscles occupy orbit. Anterior ends only of semi-circular canals formed; germinal portion thicker; cochlea slightly shorter; auditory capsule less extensively ossified.

Nerve I comes of tip of cerebrum instead of olfactory lobes. Posterior mandibular branch of V not fibrous and not closely associated with mandible.

4. Digestive. Lip groove wider than normal. Visceral slit tissue still attached to outer ectoderm.

Lung considerably smaller in bulk, with less extensive bronchioles; shaped like truncated triangle in section, conforming to abnormal shape of chest. Phrenic nerve runs at base instead of in pleuro-pericardial membrane; smaller than normal. Liver tissue more compact than normal, the sinuses less extensive; only single lobe on right. Gut slightly more coiled than normal; Meckel's diverticulum runs forward instead of back and does not enter the body cavity proper.

5. Circulatory. Tips of atria not as extensively divided. Aortic arches farther in front of lungs; right dorsal aorta persists; internal carotids complete and larger than normal; no relationship of mammary arteries to ribs, which do not meet in mid line; no separate artery to dorsal side of stomach. Extravasated blood in connective tissue of neck.

Branches of anterior cardinals somewhat larger than normal. Post cava between liver and heart about one-third size of normal. Left post cardinal persists. No direct connection between allantoic and post caval veins.

6. Excretory. Urinary ridge begins farther forward than in normal.
7. Endocrine. Oral portion of pituitary folded irregularly. Cervical portion of sympathetic smaller at anterior end.
8. Limb buds. Marginal vein still present on front foot; practically no ossification in wrist and metacarpals; bones developed in accordance with external shape. Front and hind feet more medial in relationship to rest of limbs. Only one well-ossified bone in shank region; no tendons clear. Most bones irregularly shaped.
9. Skeletal. Arches of vertebrae narrower, corresponding to shape of cord; all bones enclosing nervous system modified to fit. Ribs reach only half-way around heart; no anlagen of sternebrae, but pectoral muscles reach place where they should be.
10. Genital. No mammae present.
11. Muscles. Not able to follow out connections from sections; state of differentiation in general normal.

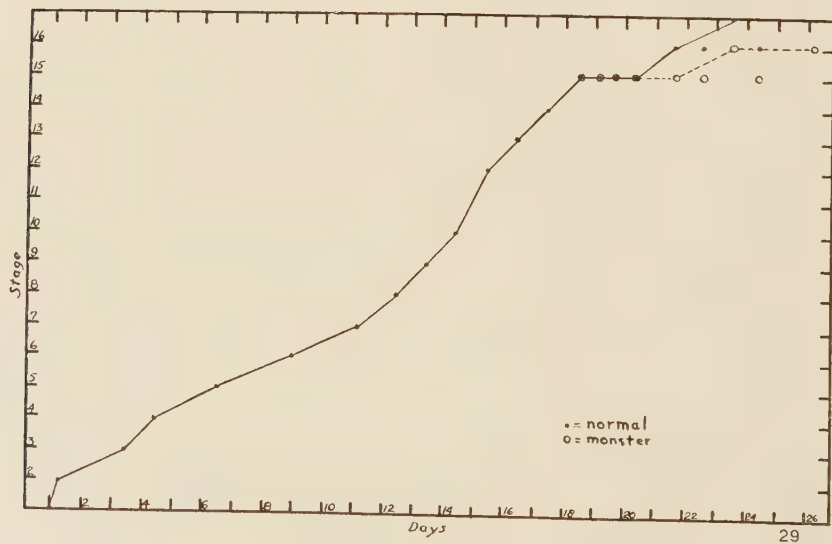
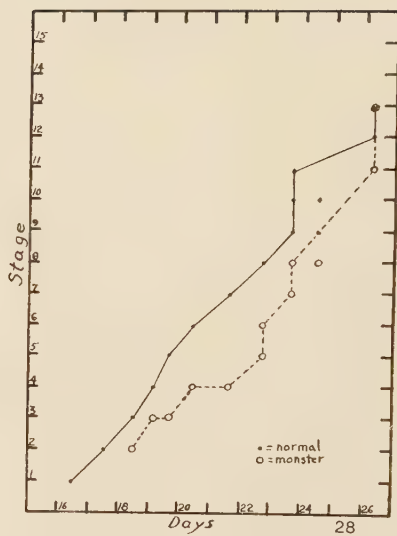
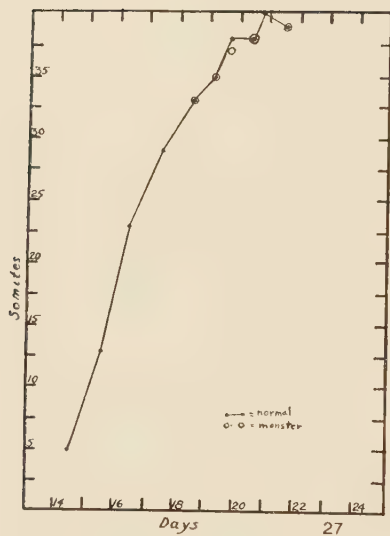
Brief comment will be made upon the nature and causal relationships of the abnormalities described above. Most outstanding is the general arrest of morphogenesis, readily apparent from the gross structure of the embryos (figs. 3 to 25). This arrest is not uniform in all organs, as can be seen from the graphs (figs. 27 to 33).³ Those organs most affected show a high rate of mitosis and excessive growth, and are the points in the embryo which were growing most rapidly just previous to the appearance of the abnormality. Those organs least affected are reduced in size, a fact more apparent in the newborn monster (Scott, '37 a) than in the embryo. On the other hand, histogenesis is much less delayed (approximately 1 day) and not unequally.

The extra-embryonic membranes and placenta are not abnormal (possibly excepting the size of the yolk sac at certain stages). The somites are almost complete at the time abnormalities appear and are also unaffected.

The neural tube is stimulated to excessive growth in the roof of the diencephalon and in the region of the cervical flexure and to a lesser extent in the region of the sacral flexure. The first prevents normal invagination of the choroid plexus and separation of the cerebral hemispheres, and by keeping the walls apart prevents the formation of the intermediate commissure. Pressure caused by this overgrowth (which does not take place in tissues surrounding the brain) may force the diencephalon outside the head and probably causes the occlusion of the aqueduct in cases of hydrocephaly. The other two regions of overgrowth result in the preservation of the cervical and sacral flexures and an arrest of changes in body conformation, which are dependent upon the growth of the neural tube.

Growth of the eye, which is attached to the ventral side of the diencephalon, is not stimulated; in most cases it is soon torn away from its attachment to the outer ectoderm and there-

³ A list of definitive stages used in preparing these graphs has been placed on file at The Wistar Institute. They may also be inferred from the author's 'Normentafel' (Scott, '37).



after all growth ceases, resulting in microphthalmia. The otic vesicle normally undergoes little development before the twenty-third day; its subsequent abnormal development is slow rather than irregular. The nerves develop slowly, sometimes pushed out of position by other aberrant organs.

An extra visceral arch (fifth) is found in the monsters, indicating some overgrowth, and the formation of the maxillary process is delayed. The small size of the eye allows the latter to occupy a dorsal position so that the two maxillae may not fuse to form the roof of the mouth, and its tip may not reach the oro-nasal groove in time to force the two wings of the premaxillary process together, thus causing harelip. The rest of the gut and the respiratory tract show relatively small delay in morphogenesis but considerable reduction in size, whether caused by lack of room for development or inhibition by the dorsal growing center.

The differences in the circulatory system, except those at the time of death, appear to be of a nature which could be produced by differences in handling and fixation or minor accidents of development, and in some cases the circulatory system of monsters is more advanced than that of the normal animals.

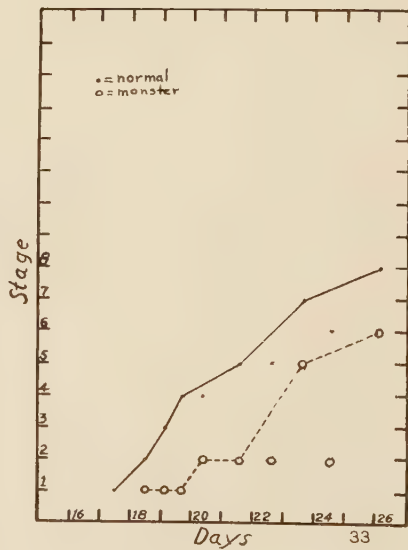
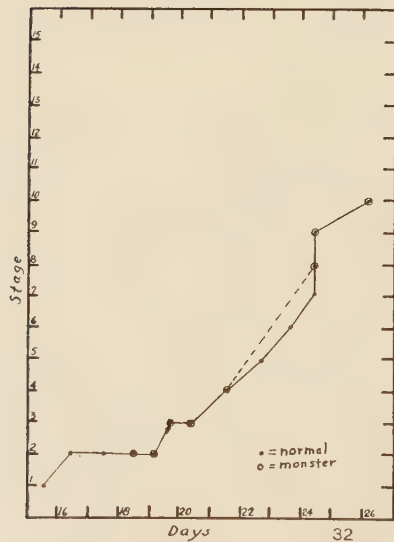
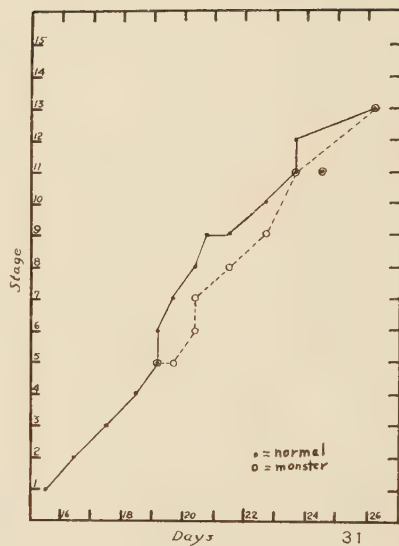
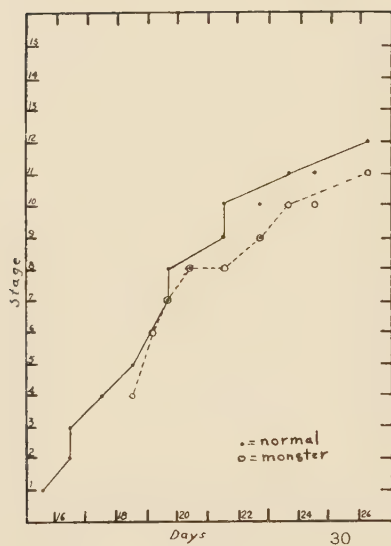
The metanephros is formed after the time of first appearance of abnormalities, but as in the ear there is some delay of morphogenesis. The differences in the genital system are probably sex differences except for the nipples, whose absence may be explained as a case of delay in a late morphogenetic process.

The skeleton is laid down to conform to adjacent organs or those in which it lies. Thus the abnormal brain produces an abnormal skull, abnormal limb buds an abnormal limb

Fig. 27 Somite numbers of monstrous and normal embryos. Compare with figure 29 and note inadequacy of this measure of development.

Fig. 28 Rate of morphogenesis of limb buds in normal and monstrous embryos. Maximum delay at about $21\frac{1}{2}$ days.

Fig. 29 Rate of change in general body form. The monsters never catch up with the normal embryos.



skeleton, and a short thorax a telescoped sternum. Bone formation is a little slow but normal. Muscle formation is likewise slow, and few abnormalities were detected in the period studied (but see Scott, '37 a).

In short, histogenetic processes (which are intracellular and directly correlated with function) are little affected, while morphogenetic processes (intracellular and not necessarily correlated with function) are greatly affected. The organ system showing functional morphogenesis (circulatory) is almost undisturbed, while that showing dependent morphogenesis (skeletal) is secondarily affected. Thus only organs which contain ectodermal and entodermal tissues are primarily affected, since mesodermal tissues do not exhibit morphogenesis by differential growth.

The induction of abortive ectodermal excrescences is curious.

THE DEVELOPMENT OF THE GUINEA PIG FOOT

Because the most lateral digits of monsters are nearly normal in appearance it has been suggested (Scott, '37 a) that there is a lateral controlling center of morphogenesis. Growth of the normal limb bud is largely produced by an increase in the amount of mesenchyme, but shape is determined by differential growth in axillary and marginal thickenings of ectoderm.

At the time of first appearance of abnormalities the limb buds have just appeared (the fore slightly more developed than the hind) and are growing rapidly. Subsequently the abnormal buds become wider and thicker but not longer than the normal, and morphogenesis is delayed (fig. 28). Secondary

Fig. 30 Rate of morphogenesis of visceral arches of normal and monstrous embryos. These organs do not show excessive growth, and delay is shorter and more uniform.

Fig. 31 Rate of morphogenesis of sense organs. Note similarity to figure 30.

Fig. 32 Stages of development of the extra-embryonic membranes. Monsters never go through certain changes in the umbilical pouch, whose development is correlated with that of the intestine. Development otherwise normal.

Fig. 33 Changes in appearance of sections through the middle of the foramen of Monro. Maximum delay at about $21\frac{1}{2}$ days. Morphogenesis of internal organs of monsters is affected in the same way as that of external ones.

ectodermal thickenings appear along the margin, similar to the normal on the lateral side but smaller and closer together on the medial. These mark the position of the future toes.

In a series of embryos from mixed strains showing no hereditary polydactyly the following sequence of events was observed. At 22-22-55 two corners of the hand marked the position of digits II and V. At 23-20-8 small ectodermal projections indicated the position of the remaining digits, that of the thumb being smaller than the rest and located at the base of digit II. Five projections had appeared on the hind foot, the three central being prominent. Skeletal thickenings were present. At 24-23-22 a projection was found at the base of digit V on the front foot. At 27-15-15, when separation of the digits had begun, this projection and those of the thumb, big toe, and little toe were found reduced to thin strips of skin. They are soon after completely resorbed along with the ridges (marginal ectoderm) between the toes.

Stockard ('30) was unable to find traces of the missing digits in embryos, but it is possible that he did not appreciate the significance (here brought out clearly in figs. 22, 24) of the small projections, which are similar to the one found normally at the base of the little finger.

Normal and abnormal development supports the theory that there is a dominant growing center in the lateral marginal ectoderm. The secondary centers of growth (projections) inhibit the formation of other centers over a certain distance, resulting in a certain number of toes being formed. Increased width of the limb bud permits the formation of other secondary centers, and increased distance from the controlling center permits release from its inhibition, causing an increasing gradient of abnormality toward the medial side. The action of the heterozygote usually does not increase the size of the foot to a point where part of it escapes from control.

The polydactylous foot is produced by increased growth plus delayed morphogenesis, complicated by the fact that most of the growth is not concerned with morphogenesis. The clubbed feet of newborn monsters represent another case of retention of embryonic posture.

SIZE AND GROWTH RATE

The above data indicate decided differences in the growth pattern of monsters, as well as interference with the type of morphogenesis dependent upon differential growth. Wright ('35) found the newborn heterozygous animals to be heavier than the normals, and one would expect the $PxPx$ embryos to be heavier than either of the other types. But the embryos were not weighed, for fear of injury, and other measurements of size must be used.

It can be seen by a glance at table 3 that there is no significant difference in crown-rump length between monsters and their

TABLE 3
Average crown-rump lengths for normal and monstrous litter mates

AGE	C.R. LENGTH IN CENTIMETERS		DIFFERENCE
	Monster	Normal	
18-12-15	0.51	0.47	0.04
19- 2-45	0.71	0.64	0.07
19-16-55	0.70	0.764	—0.064
21-15-15	0.95	1.13	—0.18
22-17-35	1.12	1.00	0.12
23-16- 5	1.53	1.51	0.02
24-13- 1	1.31	1.412	—0.102
26- 3-45	1.892	1.814	0.078
		Total	—0.018
		Mean	—0.002

litter mates. This result is misleading in that crown-rump length is dependent upon the flexure of the body, and the monsters are flexed for a longer period than the normal embryos. Table 4 shows that there is a considerable average difference in area as obtained from scale drawings, but this is still not significant with the numbers available. The monsters average 10% larger in area, which would probably mean about 15% by volume. Finally, in only three comparisons out of sixteen was a normal embryo found to be larger in area than a monstrous litter mate. Assuming that there is an equal chance of its being smaller or larger, the probability of obtaining this result is 0.025.

It is thus reasonable to conclude that there is some stimulation of growth in the monsters. The size of newborn monsters is not noticeably increased, but this may be attributed to injury at the time when the majority of monsters die.

There is no indication that the relative size of the monsters increases greatly during the period studied. Rather, the fact that morphogenetic processes which are going on most rapidly when the abnormalities first appear are most seriously affected indicates that the increase in growth *rate* is temporary and gradually disappears.

TABLE 4
Average area of sagittal sections of normal and monstrous litter mates

AGE	AREA IN SQUARE CENTIMETERS		DIFFERENCE
	Monster	Normal	
18-12-15	0.1477	0.1370	0.0107
19- 2-45	0.3300	0.2607	0.0693
19-16-55	0.2831	0.3402	—0.0571
21-15-15	0.4970	0.5581	—0.0611
22-17-35	0.6824	0.4791	0.2033
23-16- 5	0.9696	0.8148	0.1548
24-13- 1	0.7659	0.6518	0.1141
26- 3-45	1.4829	1.2874	0.1955
Total			0.6295
Mean			0.0787

The fact that monsters in retarded litters (such as 24-13-1) are of the usual type indicates that the time of increased growth is correlated with embryonic developmental processes, and tends to eliminate the possibility of an extra-embryonic cause.

CRITICAL PERIODS

There are always two important times in the development of an animal carrying a lethal factor: the time of first action of the factor and the time of death. In the *PxPx* monster there are three such periods. The onset of abnormality is first apparent at 18½ days copulation age; the lethal period for about 92% of the monsters occurs at approximately 26

days; the lethal period for the remaining 8% is the time of birth. This last 'critical period' has been described elsewhere (Scott, '37 a).

When first detectable the monsters show a state of development comparable to that of 17½-day normal embryos, and it is probable that the *PxPx* genes first act at this stage. Their primary effect appears to be stimulation of growth. Growth-promoting substances may either be admitted from the outside or manufactured in the embryo.

Outside substances must come through the allantoic placenta or the yolk sac. The former is ruled out because of two embryos in which the allantois had not become attached to the placenta, one being a monster and one normal. The latter, however, is functional at the critical period, and in the later stages the placental edge of the yolk sac of monsters is less constricted than the normal. No histological defects of the yolk sac were discovered. The possibility of the admission of a maternal hormone must be dismissed because of the irregularity of cyclical humeral function.

The hypothesis of embryonic endocrine activity is untenable. At 17½ days both the thyroid and pituitary bodies are still open diverticulae, and when the aortic bodies appear at 18½ days they are if anything less well developed than in normal embryos. None of these show any indication of function. If a growth-promoting substance is formed within the embryo it is probably produced throughout the body, perhaps entirely intra-cellularly.

The only plausible theories are: 1) admission of a maternal growth-promoting substance through the yolk sac, and 2) production of a growth-promoting substance throughout the body or in the yolk sac, probably intra-cellularly since there is no evidence of unusual secretory activity. The latter is to be preferred, since the former offers no explanation for a particular time of action.

The immediate cause of death of the monstrous embryos appears to be the bursting of small blood vessels in the region of the shoulder hump, a mass of loose connective tissue back of

the brain which is the site of a large fat body in the newborn animal. The hemorrhage spreads to other parts of the body, death resulting within a few hours. Monsters recently dead appear pale and oedematous, with patches of clotted blood beneath the skin.

Two normal events are associated with death: the appearance of large amounts of loose dermal connective tissue all over the body, and the straightening of the cervical flexure. In normal development the latter precedes the former. In addition, the dying monsters show a reduction of the post cava and of the sinuses of the liver, and the loss of a direct route through the liver by the allantoic veins. This might be caused by inadequate growth of the ventral side of the body or by retention of flexure, both of which would tend to put mechanical pressure upon the liver; or the escape of blood may lower blood pressure, resulting in small size of certain channels.

The straightening of the cervical flexure plus excessive size of the nerve cord in this region (there being no corresponding growth of surrounding mesodermal tissue) should produce considerable mechanical stress in the dorsal neck region, which may be sufficient to produce lesions, perhaps assisted by raised blood pressure. The chance of recovery would be small because the continued straightening of the flexure would increase stress.

This mechanical theory of death is supported by the fact that normal embryos are very easily bruised by handling at this period, especially around the head. A small outside pressure might be necessary to start off bleeding, the lack of which would account for those monsters which survive to birth.

DISCUSSION

The essential parts of the theory developed above to explain the action of the gene *Px* are the differential stimulation of growth at a particular time in development, and that increased growth rate inhibits the type of morphogenesis which is produced by differential growth. The facts are that the organs growing most rapidly at a particular period begin to show

excessive growth, and that these organs are subsequently delayed in morphogenesis roughly proportionally to the amount of increased growth.

Polydactylous monsters are very rare (Scott, '37 a), but most of the abnormalities are found, differently combined, in other genetically produced monsters.

The irregular polydactylous feet of Bagg's mouse strain are produced by bleb formation according to Bonnevie ('31, '34), but Plagens ('33) attributes them to clots. One or the other probably produces either mechanical isolation or physiological inhibition of the controlling center.

Landauer's ('31, '33) descriptions of the homozygous monsters produced by the Creeper fowl are at first glance strikingly similar to those of the polydactylous monster. The Creeper monsters show a dome-shaped forehead, microphthalmia, and clubbed feet. But the initial effect is a stoppage of growth at about the time of first appearance of the limb buds (Landauer, '32) followed by a later failure of histogenesis in the long bones, resulting in a chondrodystrophic condition. The Creeper monster is thus essentially unlike the polydactylous monster, and the similarities between the two can be attributed to agents acting at corresponding periods in development. It is here suggested that some of the characteristics of homozygous Creepers may be caused by retention of embryonic form.

Chesley ('35) described the development of a tailless monster in the mouse which is characterized by lateral folding of the nerve cord and failure of the underlying notochord. This folding is similar to that found in the cervical region of *PxPx* monsters, where there is no failure of the notochord. Chesley probably correctly considered the failure of the notochord to be primary, but he apparently thought of the result as an induction phenomenon. It is suggested that the similarity between the two cases is produced by opposite causes giving the same mechanical result: disproportionate growth between the nerve cord and the notochord. In the guinea pig the nerve cord is hypertrophic; in the mouse the notochord and surrounding tissues are hypotrophic.

Snell et al. ('34) have figured a type of monster occasionally obtained from the descendants of x-rayed mice which shows folding of the entire nervous system and an extreme type of flexure. No details are given, but it appears to be inhibited rather than stimulated.

Reed ('33) has described development in a race of mice producing individuals with harelip and cleft palate. He regards it as a case of local retardation of growth and morphogenesis. There is no accompanying defect of the eye, nor any indication of accelerated growth.

All of the above cases are essentially different from the polydactylous monster in that abnormality is produced by inhibition rather than stimulation. Child ('24) has stated that when development is environmentally modified differential acceleration produces a change in form opposite to that produced by differential inhibition, active regions being enlarged in one case and reduced in the other. Inactive regions develop more or less normally in both cases. This statement is correct when applied to a simple unchanging growth pattern, but where there is morphogenesis produced by a change in growth patterns it must be modified. Acceleration should preserve the original growth pattern by causing the rapidly growing portions to escape control, while inhibition prevents a change in growth pattern by stopping growth. Thus both acceleration and inhibition of growth can arrest morphogenesis in active regions.

Because the eye, digestive and respiratory tracts are reduced in size in the polydactylous monster it might be assumed that these organs were originally inhibited and that other parts of the body afterward escaped from their control and became enlarged. However, the reduced parts are not growing rapidly just before the abnormality appears, and unlike typical cases of inhibition there is no reduction in total size of the embryo. Taken together these facts rule out the application of any theory involving inhibition of growth or metabolism to the *PxPx* monster. There is, of course, an inhibition of morphogenesis.

The theory accounting for the polydactylous foot—a lateral dominant growing center which loses its control over the median side of the foot because of separation by distance—can also be reconciled with environmental experiments. The reduplicated limbs so often resulting from grafting amphibian limb buds may in some cases be caused by mechanical division of the controlling center. The fact that grafting one limb bud upon another produces a larger normal limb at first seems puzzling, but here there is no modification of the shape of the bud previous to digit formation.

Ruud ('29) was able to restore the thumb of the axolotl by grafting a fore limb bud in place of a hind one. She attributed her results to the tendency of the sciatic nerve to divide into five parts, but local conditions may have simply produced a wider bud. The latter theory seems preferable, since limbs differentiate normally in the absence of nerves. In the polydactylous monster the first effects on the limb buds are visible before nervous connections are established, and there is no apparent hypertrophy of the peripheral nerves.

The above theory is consistent with Wright's ('35) idea that multiple factor polydactyly is produced by the suppression of a digit suppressor, as the latter could be a local inhibitor of growth. But in *Pxpx* and *PxPx* animals there is almost undoubtedly a general stimulation of growth at a time when the limb buds are susceptible. The fact that heterozygous show less than half the variation from the normal found in the monsters indicates the existence of a threshold phenomenon.

Of the three unique features of the polydactylous monster—extreme polydactyly, telescoped sternum, and missing tibia—two have been explained. The condition of the tibia remains mysterious.

CONCLUSION

The physical and chemical nature of a gene cannot be established until it or its immediate products can be physically isolated. The futility of attempting to physically identify things by comparing their biological functions was originally pointed out by Owen, and the non-specific biological results

obtained by Child with known physical and chemical agents has extended the basic principle, that biological processes may be produced by a variety of agents, to include similar modification by a variety of others. The gene *Px* can be defined only biologically, but such a definition throws some light on the possible nature of primary life processes.

It is concluded that the polydactylous monster of the guinea pig is probably produced by a growth stimulating substance manufactured throughout the embryo by the *PxPx* genes for a short time after approximately $17\frac{1}{2}$ days copulation age; that the effect of this substance is greatest upon those organs growing most rapidly at the time—the limb buds, nerve cord in the cervical region, roof of the diencephalon, and visceral arches—with the result that morphogenesis is retarded in these organs proportionately to the rate of growth (the general effect being to maintain the relative growth pattern of the original period); that this results in uncoordinated development of certain related parts, eventually ending in disrupted function and death for most of the monsters between 26 and 27 days of age; that the size of certain embryonic organs thus becomes unusually large and that of others remains relatively small; and because histogenesis is uniformly and relatively little delayed complete recovery is impossible and the monster retains certain characteristics of embryonic form even if it survives till birth.

The case demonstrates that genes can accelerate as well as inhibit embryonic growth and supports the theory that an extensive modification of development is injurious, no matter what the nature of the effect. The polydactylous monster probably represents an instance of direct interference with a general process going on in all cells.

SUMMARY

1. The paper attempts an analysis of the embryonic action of the semi-dominant lethal gene *Px* in producing the polydactylous monster of the guinea pig.

2. The essential methods employed were exact timing and identical treatment of embryos, and the comparison of the most advanced normal and abnormal embryos within a litter.

3. The number of monsters obtained was consistent with genetic theory.

4. A table of differences between monsters and normals at specific ages is presented.

5. The monsters are first recognizable at $18\frac{1}{2}$ days copulation age and usually die shortly after 26 days.

6. The first visible effects are excessive growth and halted morphogenesis of those parts normally growing fastest at about $17\frac{1}{2}$ days.

7. In subsequent development morphogenesis is delayed approximately as follows: general body form, 2 days; limb buds, $2\frac{1}{2}$; sense organs and visceral arches, 1; coelom, 1; diencephalon 4; telencephalon, 2; ear, 3; gut, 1; all organs, except those of the circulatory system, to some extent.

8. The most rapidly growing organs are in general the most delayed and become most abnormal; those organs normally developing later than the critical period show delayed morphogenesis without increased growth or extreme malformation.

9. The abnormalities of newborn monsters (except for the missing tibia) are accounted for on the bases of disproportionate growth and adjustments to it, and the relatively early onset of histogenesis which 'freezes' embryonic form.

10. It is concluded that there is a controlling center of morphogenesis on the lateral side of the guinea pig foot and that polydactyly results from the excessive length of the marginal thickening which permits medial points of growth to escape from control.

11. Reduced points of growth representing the missing digits are found on the feet of non-polydactylous embryos.

12. It is concluded that delayed straightening of the cervical flexure produces an unusual strain on the loose connective tissue of that region, resulting in hemorrhage and death for most embryos.

13. It is concluded that total size of the monsters is increased over that of normal litter mates.

14. The primary visible effect of the gene *Px* is the differential stimulation of growth, inhibiting morphogenesis only where the process is one of differential growth.

15. Consequently, the polydactylous monster shows increased relative size of the most abnormal parts, whereas monsters produced by differential inhibition of growth usually show reduction of the most abnormal parts. Applied at the same time acceleration and inhibition should produce the same abnormalities but opposite proportions.

LITERATURE CITED

- BONNEVIE, K. 1931 Vererbarer Cerebrospinaldefekt (?) bei Mäusen mit sekundären Augen- und Fuszanomalien, nebst Turmschädelanlage. Abh. N. Vidsk. Akad. Oslo. Kl. J., no. 13, 26 pp.
- 1934 Embryological analysis of gene manifestation in Little and Bagg's abnormal mouse tribe. J. Exp. Zool., vol. 67, pp. 443-520.
- CHESLEY, P. 1935 Development of the short-tailed mutant in the house mouse. J. Exp. Zool., vol. 70, pp. 429-459.
- CHILD, C. M. 1924 Physiological foundations of behaviour. Henry Holt, New York.
- LANDAUER, W. 1931* Untersuchungen über das Krüperhuhn. II. Morphologie und Histologie des Skelets, insbesondere des Skelets der Langen extremitätenknochen. Zeitsch. f. mikr.-anatom. Forschung., Bd. 25, S. 115-180.
- 1932 III. The early development and lethal expression of homozygous creeper embryos. J. Genetics, vol. 25, pp. 367-394.
- 1933 IV. Die Missbildungen homozygoter Krüperembryonen auf späteren Entwicklungsstadien. Zeitsch. f. mikr.-anatom. Forschung., Bd. 32, S. 359-412.
- PLAGENS, G. M. 1933 An embryological study of a special strain of deformed x-rayed mice, with special reference to the etiology and morphogenesis of the abnormalities. J. Morph., vol. 55, pp. 151-183.
- REED, S. C. 1933 An embryological study of harelip in mice. Anat. Rec., vol. 56, pp. 101-110.
- RUUD, G. 1929 Heteronom-orthotopische Transplantation von Extremitätenanlagen bei Axolotlembryonen. Arch. f. Entwickl., Bd. 118, S. 308-351.
- SCOTT, J. P. 1937 The embryology of the guinea pig. I. A table of normal development. Am. J. Anat., vol. 60, pp. 397-432.
- 1937 a II. The polydactylous monster: a new teras produced by the genes *PxPx*. (In press.)
- SNELL, G. D., E. BODEMANN AND W. HOLLANDER 1934 A translocation in the house mouse and its effect on development. J. Exp. Zool., vol. 67, pp. 93-104.
- STOCKARD, C. R. 1930 The presence of a factorial basis for characters lost in evolution: the atavistic reappearance of digits in mammals. Am. J. Anat., vol. 45, pp. 345-377.

- WRIGHT, S. 1934 Polydaetylous guinea pigs. *J. Hered.*, vol. 25, pp. 359-362.
- 1934 a Genetics of abnormal growth in the guinea pig. Cold Spring Harbor Symposia on Quantitative Biology, vol. 2, pp. 137-147.
- 1935 A mutation of the guinea pig, tending to restore the pentadaetyl foot when heterozygous, producing a monster when homozygous. *Genetics*, vol. 20, pp. 84-107.
- YOUNG, W. C., E. W. DEMPSEY AND H. I. MYERS 1933 Some data from a correlated anatomical, physiological and behaviouristic study of the reproductive cycle in the female guinea pig. *Am. J. Physiol.*, vol. 105, pp. 393-398.

